Genetic Structure, Genetic Diversity and Inbreeding in Reintroduced Alpine Ibex (*Capra ibex ibex*) Populations

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Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

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Zürich, 2009

Die vorliegende Arbeit wurde von der Mathematisch-naturwissenschaftlichen Fakultät der Universität Zürich im Frühjahrssemester 2009 als Dissertation angenommen.

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GENERAL INTRODUCTION

Risk of small populations

All populations fluctuate in their size due to temporal random changes in birth, death and growth rates. The main reasons for temporal changes in these vital rates come from environmental stochasticity, demographic stochasticity, catastrophes and bonanzas (Morris & Doak 2002). While such stochastic fluctuations do not threaten large populations, they constitute an increased extinction risk for small populations (Soulé 1987). For example, if there are only few individuals in a population left, they might all be hit by an environmental catastrophe or affected by a disease, while in large populations chances are higher that some individuals survive. In addition, at small population sizes genetic effects such as inbreeding and genetic drift may contribute to the extinction of a species. Synergistic interactions between population growth rate, genetic effects and impact of random demographic and environmental events may lead to populations constantly decreasing until extinction, a concept known as the extinction vortex (Gilpin & Soulé 1986; Soulé 1987). Thus, environmental, demographic and genetic stochastic effects can all, either individually or in combination with each other, constitute a potential risk to small populations (Soulé 1980; Frankel & Soulé 1981).

Anthropogenic actions have reduced an increasing number of species to populations of small size due to habitat destruction, habitat fragmentation and overharvesting. In recent years, numerous conservation programmes have been initiated with the purpose to reverse the decline of populations and species by restoring habitat, augmenting populations with individuals from other populations or founding new populations by reintroductions (Short *et al.* 1992; Jones *et al.* 1995; Spalton *et al.* 1999; Ralls *et al.* 2000; Armstrong *et al.* 2006). The basic logic behind all these programmes is to create a rapid increase in population size so that the populations are not threatened by stochastic fluctuations any more. However, while environmental and demographic stochasticity only depend on the current population size, genetic stochasticity (e.g. inbreeding) also depends on past population sizes. Thus, contrary to environmental and demographic risks,

genetic risks can still be substantial even after a population has been protected from the immediate threats that caused its decline.

During the time when populations are small, they experience increased genetic drift that leads to loss of genetic variation and inbreeding. Loss of genetic variation reduces the ability to adapt to changing environments (James 1970; Allendorf & Luikart 2007). Though the ability to adapt to a changing environment is thought to be of relevance only in the long-term, it may gain importance as the environment is changing faster due to human induced changes e.g. in the climate. However, there is also a more immediate risk, the reduction of fitness due to inbreeding (inbreeding depression). An increasing number of studies suggest that the magnitude of inbreeding depression in natural populations can be substantial in wild populations of animals and plants (e.g. Crnokrak & Roff 1999; Keller & Waller 2002; Reid *et al.* 2003; Ivey *et al.* 2004; Hogg *et al.* 2006; Fredrickson *et al.* 2007). Furthermore, interactions between inbreeding depression and the environment have been reported in many species with higher inbreeding depression under stressful environmental conditions (e.g. Coulson *et al.* 1998; Coltman *et al.* 1999; Bijlsma *et al.* 2000; Keller *et al.* 2002; Ross-Gillespie *et al.* 2007). Such interactions may constitute an increased risk for some species, because habitat destruction is often the root cause of the decline and thus the environment may already be stressful for these species.

It is for the above reasons that understanding how bottlenecks affect genetic structure and genetic diversity is important in conservation biology, as are investigations of ways to maintain genetic diversity and reduce inbreeding in managed species. Addressing these issues requires ideally several populations of the same species that differ in their population history. Reintroduced species often provide such a set-up because they have experienced bottlenecks due to founder events and if several populations have been reintroduced they may differ in the founder composition and growth rate after the founder events. While there are numerous species that have been reintroduced to several locations, studies with detailed knowledge of the population history in combination with genetic data are scarce (Latch & Rhodes 2005; Taylor & Jamieson 2008).

Alpine ibex populations in Switzerland are exceptional, because the population history since founding of the populations has been well documented. Additionally, the fact that several populations descended from one common ancestral population makes them well suited to an investigation of inbreeding depression at the population level. Most studies so far have only studied inbreeding depression at the individual level, but not at the population level (Keller *et al.* 2007). We still know little about the effects of inbreeding depression on population dynamics, yet understanding inbreeding effects at the population level is crucial, for both evolutionary and for conservation biology (Keller & Waller 2002; Keller *et al.* 2007). If inbreeding depression in individual fitness translates into reduced population growth rates it is of immediate conservation concern for inbred populations. However, if there are positive population growth rates despite high inbreeding levels, there are two possible scenarios leading to different long-term evolutionary predictions (Hedrick 2001). Either growth rates are not affected despite apparent inbreeding depression at the individual level because of soft selection or the genetic load has been purged.

Investigating inbreeding at the population level may have additional advantages. Inbreeding depression might be difficult to detect if it is measured within a population with random mating because there might not be sufficient variation in inbreeding levels between individuals (Hedrick & Kalinowski 2000). In the extreme case, a population may be fixed for its load of deleterious alleles and therefore there are no fitness differences among individuals of different inbreeding levels. However, comparisons between two or more inbred populations might reveal fitness differences in such situations. However, this requires measuring inbreeding and fitness at the population level. Nearly fixed loads of deleterious alleles in populations with a history of small size have also been detected with a genetic rescue effect following the immigration of unrelated individuals (Westemeier *et al.* 1998; Madsen *et al.* 1999; Hogg *et al.* 2006). Three inbred wolf lineages are another example where no inbreeding depression was apparent within lineages, however offspring of crossed lineages had higher probabilities of live birth and higher litter sizes (Fredrickson *et al.* 2007).

Measuring inbreeding

To study the effects of inbreeding one obviously needs to quantify the levels of inbreeding in a population. This is no trivial task in natural populations (Keller & Waller 2002). There are two general approaches to measure inbreeding in natural populations: Pedigree analysis and molecular genetic analysis. Most recent studies of inbreeding depression in wild animals use genetic markers to estimate inbreeding because pedigree analysis requires accurate parentage information over several generations, which is rarely available for wild populations (Keller & Waller 2002). To estimate inbreeding at the individual level in the absence of pedigree data, individual heterozygosity is widely used (Coltman & Slate 2003). However, theoretical models and comparisons of inbreeding coefficients from pedigree with genetic data in domestic and wild populations suggest that individual heterozygosity from neutral markers correlates only weakly with the inbreeding coefficient (Bierne *et al.* 2000; Balloux *et al.* 2004; Slate *et al.* 2004; DeWoody & DeWoody 2005; Aparicio *et al.* 2007), unless many markers are used or there is substantial identity disequilibrium. The latter occurs for example when inbreeding is caused by non-random mating (e.g. selfing). However, on a population level the picture is different. Microsatellite heterozygosity and nucleotide diversity in non-coding regions often correlate at the population level even when such a correlation is absent at the individual level (Vali *et al.* 2008). Therefore, inbreeding can be estimated with reasonable accuracy using data from genetic markers at the population level.

Following (Jacquard 1974) we can define inbreeding at the population level as follows: "*The inbreeding coefficient* α *of a population is the probability that the two genes of a member of the population taken at random are identical by descent*". In populations with random mating Wright`s Fst statistic measures inbreeding according to this definition (Wright 1965, p. 407). Vitalis (2001) and Weir & Hill (2002) discuss issues related to the estimation of this quantity. An alternative approach to estimate the average rate of population inbreeding is the estimation of effective population sizes (Keller & Waller 2002). This approach is a

useful addition to population specific Fst as it refers to a different time scale and makes different assumptions.

"Genetic structure, genetic diversity and inbreeding in reintroduced Alpine ibex (*Capra ibex ibex*) populations": This Thesis

In this thesis I addressed the genetic consequences of populations that experienced bottlenecks or founder events in reintroduced wild populations of Alpine ibex. The history of the Alpine ibex populations in Switzerland, with its near-extinction in the 18th century and the following successful reintroduction, represents a large-scale genetic experiment. All populations have one common ancestral population and therefore inter-population relationships are solely a function of founding histories and intrinsic populations dynamics following the founding event. Relationships are not confounded by gene flow as Alpine ibex exclusively occupy high Alpine habitats, and little if any exchange occurs among populations from disjunctive mountain ranges (Nievergelt 1966). Complete histories of the number of founder individuals, the source of the founder individuals and yearly census sizes were available for many populations. Here I used this independent information of the reintroduction history, together with molecular genetic data, to infer the influence of historical events on population structure, genetic diversity, inbreeding levels and inbreeding depression.

Study Species and System

All nine ibex species of the genus *Capra* (wild mountain goats) have in common that they live in rocky habitat with extreme climate (Shackleton & Group 1997) in Eurasia, North Africa and East Africa. Humans hunted most ibex species, partly to get meat, but also because parts of the body were supposed to have healing and aphrodisiac properties. As a consequence of the hunt most ibex were decimated and driven nearly or, as in the case of two subspecies of the Spanish ibex completely to extinction (Perez *et al.* 2002). Owing to the Italian king Vittorio Emanuelle III (1900 - 1946) Alpine ibex went not extinct, because a small Alpine

ibex population of less than 100 individuals (Grodinsky & Stuwe 1987) survived in his private hunting reserve in northern Italy in the Gran Paradiso region. He employed game keepers to protect the Alpine ibex in his hunting reserve from poaching so that he could indulge in his passion. So ironically, the same activity that drove all other Alpine ibex populations to extinction, saved the species from complete disappearance on earth (Giacometti 2006).

Following extirpation from Switzerland, the Swiss made an effort to resettle Alpine ibex in their country at the turn of the 20th century. After failed reintroduction attempts with F1 hybrids between Alpine ibex and domestic goat, the Swiss tried to obtain pure Alpine ibex (Giacometti 2006). The first pure Alpine ibex were illegally taken from the last remaining population in Italy and smuggled to Switzerland, because Vittorio Emanuelle III refused permission for exporting Alpine ibex from his reserve (Giacometti 2006). Animals were bred in two zoos prior to first reintroductions into wild habitat. Further reintroductions from a few established wild populations re-established Alpine ibex across Switzerland. Many of the reintroduced Alpine ibex populations increased rapidly in size, and controlled hunting started again in 1977 in populations with high density (Giacometti 1988). Generally the number of individuals that are hunted in autumn is determined from the census count in springtime. However, in the recent years some of the populations decreased in size for unknown reasons. A national Alpine ibex project was launched by the FOEN (Federal Office for the Environment) to gather information about causes for the decline. The project includes a population dynamic, disease and genetic module, and this thesis is covering part of the genetic module.

Sampling

I collected genetic samples from populations across Switzerland, and additionally I had access to samples from the Gran Paradiso National park, the ancestral population. The main part of the sampling was done by game keepers that sent us a small piece of tissue of each shot animal. However, there are also populations without hunting or with too few animals hunted to get a sufficient

sample size. Some of these populations were small and declined in size, and in order to have the range from large to small populations in the analysis, these were especially interesting to us. Sampling options in these protected populations were anaesthetizing animals to take blood, collecting faeces or using biopsy darts.

I did not anaesthetize Alpine ibex because of the risk that the animal falls to death in the steep habitat during the time until the narcotic substance is effective. Though it is possible to get genetic data from ibex faecal samples (Maudet *et al.* 2004, pers. obs.), they yield only low quality of DNA and therefore time and financial costs for laboratory work are higher than for high quality DNA. Furthermore, the effort in the field of collecting faeces is similar to sampling with biopsy darts, and thus we used the latter method. Biopsy darts (Karesh *et al.* 1987) punch out a small piece of skin about 30 mm^3 from the animals. The biopsy dart (Figure 1) was shot from a maximum distance of 25m with a CO_2 injection rifle (Model JM Special; Dan-Inject) on the animal and bounced immediately off. I built my own darts by modifying the original model for our purposes. I shortened the length of the whole dart and the length of the front cutting projectile to 5 mm such that usually only skin and little fat were punched. Two barbed nerve-broaches from the dentist equipment take the piece of skin when the dart is falling off. Using this method I collected 108 samples from 6 protected populations and from populations where only few animals are hunted. To avoid sampling of the same individual twice I attempted to recognize each individual by characteristics of their horn morphology.

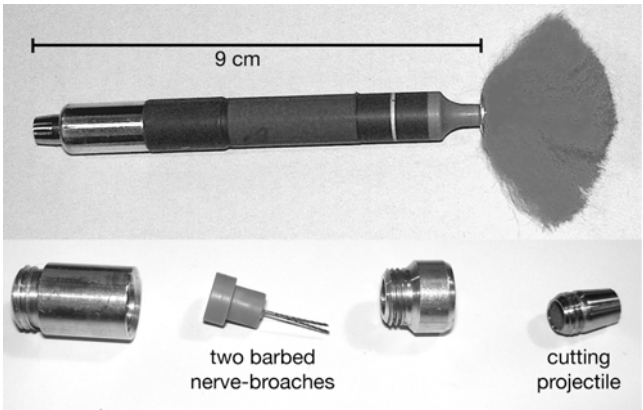

Figure 1: Biopsy dart that was used to sample protected populations

Outline of the thesis

In **Chapter 1** of this thesis I study the genetic structure and genetic variation of the Alpine ibex populations in Switzerland with respect to their history. I used information on their reintroduction history to investigate its effect on the genetic pattern at present. Additionally I assessed the effect of the number of serial founder events on the loss of genetic variation. Genetic variation was measured with expected heterozygosity and number of alleles per population, because they are differentially affected by the bottleneck size and population growth rate following the founder event.

Chapter 2 deals with the impact of the founder group composition on genetic variation in reintroduced populations. Using information on the number and the origin of the released individuals per population. I investigated the relative effect of founder group size and admixture of the founder group on genetic variation in the Swiss Alpine ibex populations. Measures of genetic variation were the same as in Chapter 1, so expected heterozygosity and number of alleles. Furthermore I estimated the number of genetic founders to explore the proportion of released individuals that survived. I used both these genetic founders and the actual number of animals released to estimate the effect of founder group size on genetic variation.

Population history affects the levels of inbreeding. Understanding the impact of demographic history on inbreeding is the goal of **Chapter 3**. I estimated inbreeding for three different time scales. I used population specific Fst to estimate the accumulation of inbreeding relative to a common ancestral source. By choosing populations with different common ancestral sources I estimated inbreeding over the whole time period since ibex were brought to Switzerland and over the time since populations were founded by a second founder event. Inbreeding that is generated each generation at present in the ibex populations was evaluated on the basis of their effective population size. I investigated the impact of demographic history such as founder group size, admixture in the founder group, harmonic mean population size since founding and census size on these inbreeding estimates.

Although our understanding of how inbreeding affects individuals has improved, we still know little about the effects of inbreeding on population dynamics. Therefore, in **Chapter 4** I related inbreeding at the population level to the deterministic growth rate and density dependence of populations. I calculated inbreeding estimates relative to the zoo populations (Chapter 3) and estimated growth rates and density dependence with a state-space model that fitted a logistic population model to the data. In addition to the effects of inbreeding levels I studied the effect of hunting intensity on the two parameters, population growth rate and density dependence.

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1 A STRONG GENETIC FOOTPRINT FROM THE REINTRODUCTION HISTORY IN ALPINE IBEX (*CAPRA IBEX IBEX*)

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Submitted to Molecular Ecology

Abstract

Genetic structure of neutral markers among populations is a composite of demographic events that populations experienced in their history. Each bottleneck creates genetic drift with loss of genetic diversity and increased genetic differentiation in relation to other populations. However, gene flow and admixture override the influence of founder events on genetic structure. With detailed knowledge of the demographic history of 42 reintroduced Swiss Alpine ibex populations we investigated possible influence of gene flow on genetic structure and studied the effects of serial bottlenecks on genetic variation using 37 neutral microsatellites. A strong footprint of the reintroduction history was evident in the today's genetic structure. Genetic variation among populations was more than two thirds influenced by reintroduction history. Due to the translocation of many individuals most of the genetic variation of the ancestral population was brought to Switzerland. However, genetic variation was subsequently divided up such that each Swiss population now has lower genetic variation than the ancestral population. Serial bottlenecks had differential effects on the two measures of genetic variation, expected heterozygosity (He) and number of alleles (Na). While loss of Na was higher in the first bottleneck than in subsequent ones, He declined by the same amount with each bottleneck. Thus, there was genetic drift with each bottleneck, even when no loss of Na was observed. These findings provide important information for future reintroduction programs and conservation and management of small populations.

Introduction

Most populations fluctuate in size over time, and harsh climate conditions or natural catastrophes can result in local reductions to small sizes or even extinction (Morris & Doak 2002). However, often populations recover from the bottleneck and new populations are established by immigration of individuals from neighbouring populations (Grant *et al.* 2001; Keller *et al.* 2001). Human activities have however increased the number of populations being reduced or extirpated. Due to fragmentation of habitats the time until empty habitats are occupied by dispersing individuals has increased, if dispersal is possible at all (Colas *et al.* 2004). Therefore reintroduction programs have become an important tool in restoring and augmenting wildlife populations (Griffith *et al.* 1989).

While the abundance of a species might be as high as before the reduction period, the original genetic variation and structure is however rarely restored. During bottlenecks and extirpations (local extinctions) genetic diversity is lost that might not be represented in other populations. Also, during the recovery phase each founder event leads to genetic drift with additional loss in genetic diversity and increased genetic differentiation between the founder and source population. Genetic differentiation and loss of genetic variation due to founder events or bottlenecks is seen in many natural populations (e.g. Keller *et al.* 2001) and reintroduced populations (Scribner & Stuwe 1994; Groombridge *et al.* 2000; Hedrick 2001; Williams *et al.* 2002). Furthermore simulations of serial founder events show an increased effect of fixations of alleles and genetic differentiation (Le Corre & Kremer 1998). Thus while already one severe bottleneck can lead to substantial differentiation (Chakraborty & Nei 1977; Hedrick 1999) it can be amplified in serial founder events and drastically alter the genetic structure of natural populations (Pruett & Winker 2005).

The fact that history is shaping genetic structure can be used to infer the history of populations. Reconstructing dispersal routes (Lorenzen *et al.* 2006; Schug *et al.* 2007) or finding the source of introduced populations (Goodman *et al.* 2001; Gaskin *et al.* 2005; Kolbe *et al.* 2007), are only few examples of this. Unfortunately however recent demographic events can override the effect of

historical factors on population genetic structure (Johansson *et al.* 2006) and obscure a clear picture of the history (Kuehn *et al.* 2004). Thus the genetic structure of neutral genetic markers we find at present in populations is a composite of the demographic events experienced by populations across their complete history. The effect of founder events on genetic population structure depends on the time since it occurred (Nei *et al.* 1975) as well as on the intensity of gene flow from admixture with neighbouring populations (Wright 1931). Therefore, when we know the history of reintroduced populations, we are able to test whether there has been gene flow after founder events. Finally, defining the genetic differentiation and variation of wild animal populations is essential for determining the appropriate scale of their conservation and management (Moritz 1999; Allendorf & Luikart 2007).

Genetic drift in small populations leads to both genetic differentiation and loss of genetic variation. In ideal populations with finite population sizes expected heterozygosity (He) is lost at a rate proportional to 1/2N (Gillespie 2004), where N is the population size. Thus, founder events have a substantial effect on loss of He due to the low founder group size. The population growth rate after the founder event is a further determinant of loss in He. The loss of number of alleles (Na) during a founder event is not only determined by the size of the bottleneck but also by the number and frequencies of the alleles present (Kimura 1955; Allendorf 1986). Populations in mutation-drift equilibrium have a considerable number of alleles at low frequencies (Nei 1987). Given a certain bottleneck size, more alleles are lost the higher the number of alleles present and the higher the proportion of alleles at low frequencies before the bottleneck, because alleles at low frequencies are lost preferentially (Maruyama & Fuerst 1985). Therefore, in contrast to He the loss in Na is a function of the genetic variation in the population before the bottleneck and thus serial bottlenecks are expected to have different effects on He and Na.

Alpine ibex is a species that experienced several bottlenecks during the reintroduction history. Alpine ibex were successfully reintroduced into most parts of the Alps resulting in many populations that are all descending from one

ancestral population in northern Italy. Reintroduction history is well known and therefore there are replicated reintroduced populations with a known number of experienced bottlenecks. Populations have been reintroduced densely across Switzerland such that gene flow between many populations is possible after populations have increased in size. We used 42 ibex populations of this system to study the effects of the number of experienced founder events on genetic variation and to investigate genetic structure in relation to the reintroduction history by using neutral microsatellites.

Methods

Alpine ibex reintroduction history

Alpine ibex almost went extinct in the 18th century due to overhunting, but reintroductions from the sole remaining population in the Gran Paradiso region in Italy have re-established populations across a large part of the European Alps. Nowadays, 40 000 ibex live again in the Alps, 14 000 of which in Switzerland (Shackleton & Group 1997). During the decline of Alpine ibex the ancestral population experienced the first known bottleneck when the population was reduced to less than 100 individuals (Grodinsky & Stuwe 1987). During the reintroduction ibex populations in Switzerland experienced up to four further bottlenecks caused by founder events (Figure 1): The first additional bottleneck took place when about 100 ibex were transferred from the Gran Paradiso population to Swiss zoos between 1906 and 1940 for a captive breeding program. Four zoos bred ibex but only two were the main sources for establishing the first populations in natural habitat from captive animals, causing the second bottleneck. The size of the captive founder stock is estimated at 88 individuals (Stuwe & Nievergelt 1991). The third bottleneck occurred when three of these captive-founded populations, Mont Pleureur (pl), Albris (al) and Brienzer Rothorn (br) served as the main source populations for most populations founded since then in Switzerland, Germany and Austria. Some populations, in turn, were established with animals from these wild-founded populations leading to an additional, fourth bottleneck. Most of these reintroductions are well documented

with known origin of founder animals (Couturier 1962; Stuwe & Nievergelt 1991) (Table 1).

Samples and genetic data

For genetic analysis we collected 1262 ibex samples across Switzerland and from the Gran Paradiso region in the years 2003-2007 (Table 1). Samples consisted of tissue or blood obtained from legally hunted, naturally deceased or anesthetised animals and from collection with biopsy darts. Tissue samples were stored in 100% Ethanol and blood samples in APS buffer at -20°C before genomic DNA extraction with a commercial kit (BioSprint 96 and QIAamp DNA Mini Kit; QIAGEN).

To avoid circularity we deliberately did not use information from genetic data to define populations. Instead we used information on likely dispersal barriers obtained from game wardens to define population boundaries, resulting in 42 populations with sample sizes from 17 to 61 (Table 1). The 42 populations included the ancestral Gran Paradiso population, 3 captive-founded, 31 wild-founded and 7 mixed-founded populations. Captive-founded populations include populations that were founded with animals from the captive breeding enclosures only. In addition we included one population (pl) in this category, which mainly received animals from captivity but also six directly from the Gran Paradiso population. Wild-founded populations were defined as populations with founder individuals from already established natural populations in Switzerland and mixed-founded populations received animals from both, captive and wild Swiss populations (Figure 1; Table 1).

When comparing reintroduced populations to their sources, we compared the three initial captive-founded populations to the ancestral Gran Paradiso population, even though at first sight the original population in Swiss zoos would be the correct comparison. However, we only had access to recent samples from the zoos and they no longer represent the gene pool of the founder individuals due to genetic drift and transfers of animals in the intervening time. Since we

were interested in the loss of genetic diversity caused by serial founder events, the ancestral Gran Paradiso population is thus the better choice for comparison purposes. Census size of the Gran Paradiso population has been between ~3000 to ~5000 (Maudet *et al.* 2002; Jacobson *et al.* 2004) since reintroduction of animals into Switzerland and thus we do not expect substantial changes in allele frequencies due to drift. However, the ancestral population does not represent the historic levels of genetic diversity in Alpine ibex since the Gran Paradiso population also experienced at least one bottleneck.

We genotyped all samples for 44 microsatellite loci originally isolated in domestic cattle, goat and sheep, which were known from the literature to amplify and to be polymorphic in Alpine ibex (Maudet *et al.* 2001; Maudet *et al.* 2002; Maudet *et al.* 2004; Von Hardenberg *et al.* 2007). PCR was conducted in 10 independent multiplex reactions (QIAGEN) co-amplifying up to eight microsatellites within each reaction. PCR products of 30 to 36 amplification cycles depending on the DNA concentration were pooled into six different fragment analysis runs on an ABI 3100 Avant automated sequencer. Allele sizes and genotypes were determined using the software GENEMAPPER 3.7 (Applied Biosystems) and LIZ size standard followed by manual proofreading. Unreliable genotypes were repeated once and discarded from further analysis if the repetition did not yield reliable genotypes. Four microsatellite loci (ETH10, OarKP6, BM1258, BM1818) that are known to be possibly under selection (Paterson & Crawford 2000, Von Hardenberg *et al.* 2007, NCBI Map Viewer) were omitted from further analyses. Details about the microsatellite loci and PCR conditions are given in Appendix 1. To estimate the frequency of genotyping errors, 7.5% of the samples per locus were additionally repeated.

Data Analysis

(i) Genetic diversity

We estimated allelic dropout and false allele rates with a maximum-likelihood-based method implemented in PEDANT (Johnson & Haydon 2007). Loci possibly

under selection can severely bias estimates of population parameters (Allendorf & Luikart 2007). To identify such loci, we assessed deviations from the neutral expectation with the program FDIST2 thinning out populations with correlated allele frequencies as described in Beaumont & Nichols (1996). Deviations from linkage equilibrium (LD) between pairs of loci within populations and from Hardy-Weinberg equilibrium (HWE) for each locus within populations were estimated with the program ARLEQUIN version 3.1 (Excoffier *et al.* 2005). LD was assessed with a likelihood-ratio test using 10,000 permutations. Fisher's exact tests were performed to test for significant deviations from HWE using a Markov chain of 100,000 steps and 1,000 dememorization steps. We corrected the p-values for multiple comparisons with Holms' method (Holm 1979).

We examined two measures of genetic variation, expected heterozygosity (H_e) and average number of alleles (Na). Measures of genetic variation were calculated in ARLEQUIN for each locus and population and also averaged over all loci for two groups, the ancestral Gran Paradiso population and all Swiss populations, to examine if genetic variation was retained in the source population that is not existent in reintroduced Swiss populations. We estimated Na using the absolute allelic richness values and not values standardized for different sample sizes per population. Though differences in sampling intensity can bias allelic richness (Leberg 2002), we believe that our results will be less biased without standardization for the following reasons: First, all Swiss populations experienced bottlenecks and therefore the influence of sample size on Na is less pronounced and the asymptotic level of Na is reached with fewer samples (Leberg 2002). In fact, asymptotic Na was reached for each population at sample sizes below the actual one in our dataset (data not shown). Second, we sampled different proportions of the populations, something not taken into account by standardization methods. Thus, we chose not to standardize estimates of allelic richness for sample size. However, results did not change when using standardized allelic richness values calculated with the rarefaction method (Petit *et al.* 1998) in FSTAT Version 2.9.3.(Goudet 2001). Furthermore a subset of Na, the alleles at frequencies below 0.05 (rare alleles) were examined, because they are expected to be preferentially lost during bottlenecks (Maruyama & Fuerst 1985).

To assess the effects of reintroduction history on current population structure, we classified populations as being descendent from one of the three initial captive-founded populations. Because these three initial captive-founded populations were among the first wild populations re-established during the reintroduction programme, we call them 'historical groups', e.g. the pl-group with its descendant populations is one historical group (Figure 1). We partitioned the genetic variation between individuals within populations, among populations within historical groups (al-, br- and pl-group) and among historical groups with a hierarchical Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) in ARLEQUIN. Populations were assigned to one of the three historical groups according to the source of the founder individuals. To preserve the strict hierarchical structure required for an AMOVA, we only included populations in this analysis where all founder animals originated from one of the historical groups (see Table 1).

(ii) Serial bottlenecks

The effects of serial bottlenecks on loss of genetic variation were assessed for two categories of populations that differed in the number of bottlenecks they experienced. The three captive-founded populations that experienced two serial bottlenecks represent one category and 21 populations that experienced three to four serial bottlenecks form the second category (Table 1). We assigned populations to the second category if they were wild-founded and belonged to one historical group. We could not distinguish between three and four bottlenecks, because there were many populations that received animals from the captive-founded populations and from already established wild-founded populations and thus their founders had experienced both three and four bottlenecks. For the first category the source population was the gp population and for the second category the corresponding captive-founded population was the source population. Differences in He, Na and rare alleles between the populations and their sources were tested with an analysis of covariance with number of bottlenecks (one to two or three to four bottlenecks) as factorial

explanatory variable and the natural logarithm of number of released individuals as continuous explanatory variable. We included number of released individuals because bottleneck size also affects the loss of genetic variation (Wright 1951).

(iii) Genetic structure

We quantified genetic structure and differentiation among all populations using pairwise Fst (Weir & Cockerham 1984) and Nei's D (Nei & Li 1979) with ARLEQUIN and 10,000 permutations for significance. We did not use Rst because the time scale of interest in these analyses was ten to twenty generations and mutations therefore can be neglected (Slatkin 1995). We generated an unrooted, neighbour-joining tree using MEGA version 4.0.2 (Tamura *et al.* 2007) based on pairwise Nei's D.

We also asked whether the reintroduction history was evident in today's genetic structure without a priori knowledge of population history. To that end, we inferred genetic structure with a Bayesian clustering method implemented in STRUCTURE version 2.0 (Pritchard *et al.* 2000). This method assigns genotyped individuals probabilistically to one or more clusters (K) assuming HWE and linkage equilibrium within each cluster. Posterior probabilities of the data given a specified K are estimated. We set K from 1 to 12, and chose the correlated allele frequencies with admixture model, because our populations are closely related due to the common ancestry and are likely to be admixed either through natural migration or translocations by humans. Five repetitions for each K were run with 200,000 iterations and a burn-in period of 50,000 iterations.

All statistical analyses were carried out with the R package, version 2.8.0 (R Development Core Team 2006).

Results

Genotyping errors, HWE and LD

Allelic dropout rates per genotype were below 0.075 for all loci, but the majority of the loci (73%) had allelic dropout rates below 0.01. False allele rates per genotype were below 0.01 for all loci (Appendix 1). FDIST2 results suggested that two microsatellite loci might be under selection, BM4208 exhibiting less and OarHH62 more differentiation than expected for a neutral locus. After correction for multiple testing SR-CRSP07 showed significant excess of homozygotes in 44% of the populations, suggesting the presence of null alleles. Thus this marker and the two markers possibly under selection were removed for further analysis. Six additional microsatellite loci (BM1225, BM4208, OarFCB193, OarHH35, SR-CRSP23 and SR-CRSP24) deviated significantly from HWE in one population each (gl, sm, pg, gp, gp and go respectively), all exhibiting an excess of homozygotes. If null alleles caused these deviations, we would expect a higher proportion of populations out of HWE. These deviations, therefore, more likely reflect recent immigration or substructure within the defined population. However, no population had more than two microsatellite loci that were not in HWE, suggesting no overall Wahlund effect in the defined populations. Significant linkage disequilibrium was detected in 1.8% of the pairwise comparisons after correction for multiple tests. Pairs of loci that are on the same chromosome on the bovine genetic linkage map (NCBI Map Viewer) were not more likely to be in significant LD than pairs of loci on different chromosomes (fishers-exact test: p=0.07), although there was a trend in the predicted direction. Thus, the observed LD is probably the consequence of the migration and genetic drift caused by the translocations (Gillespie 2004). We did not exclude from further analysis the microsatellite loci that exhibited LD, as many summary statistics such as Fst are robust to LD (Weir & Cockerham 1984).

Of the apparently neutral 37 loci that remained in the dataset on average 36.8 loci were genotyped successfully across all samples.

Genetic variation and effects of serial bottlenecks

In accordance with previous studies (Stuwe & Scribner 1989; Maudet *et al.* 2002) Alpine ibex exhibited low genetic variation (Table 1). Mean He was 0.41 (range 0.34-0.47) and mean Na 2.5 (range 2.2-3.1) over all loci and all populations**.** He and Na varied up to 29% and 37%, respectively, among populations and they were significantly correlated ($r=0.79$, $n=37$, $p<0.001$) suggesting that Na explained approximately 60% of the variation in He.

The ancestral gp population had the highest number of alleles and together with the gl population the highest proportion of alleles with a frequency below 0.05 (Table 1). Yet, taken together, all Swiss populations had a similar or higher genetic variation (He=0.45, Na=3.5) than the ancestral gp population (He=0.45, Na=3.1).

To both categories of populations experiencing a different number of serial bottlenecks on average 46 individuals were released. Accordingly, the number of released individuals did not have a significant effect on either of the tested response variables, loss of Na, loss of He and rare alleles. Loss of Na and rare alleles between source and founder population were significantly higher for the first category than the second category (Table 2). About half of the lost alleles (46%) were rare alleles for the populations experiencing one to two bottlenecks. The loss of He was not different between the two categories, both had between 0.02 and 0.03 lower He in the founded population compared to the source population. As a consequence the two categories had different absolute levels of He because they had experienced different numbers of bottlenecks. The first category had 0.43 He and the second category 0.39.

Influence of reintroduction history on genetic differentiation and structure

We found highly significant ($p<0.001$) genetic differentiation at every hierarchical level, with 9.8% of the genetic variation occurring between the historical groups, 4.5% among populations within the historical groups and 85.7% within populations.

The cluster analysis revealed that the dataset comprised between 3 to 11 clusters. The log probability of the data increased only slightly above K=3 and reached a plateau above K=11. At K=3 85% of the individuals have a proportion of membership to one of the clusters above 0.8, while for higher K only 62% or less of the individuals are assigned obviously to one cluster. Thus, we show the summary per population for three clusters (Figure 2), as K=3 is the smallest value capturing the major structure (Pritchard *et al.* 2007). The three clusters clearly reflected the early reintroduction history of Alpine Ibex: Each cluster comprised one of the captive-founded populations (al, br and pl) and their descendant populations. The ancestral population has mixed assignment probabilities to the three clusters, 52% to the br cluster, 29% to the pl cluster and 19% to the al cluster. The mixed founded populations had highest assignment probabilities to one of the sources of their founder individuals from the wild populations.

In the pairwise comparisons of genetic differentiation all but the one between js and vb were significant at the 5% level. Fst values of significant comparisons ranged from 0.005 (al-av) to 0.223 (fl-ob). Average pairwise Fst between the three captive-founded populations and the ancestral gp population were significantly higher (mean and standard error: 0.087±0.007) than between wild-founded populations and their source (mean and standard error: 0.032±0.003) considering only wild-founded populations that descended from a single source (t-test: $t=5.69$, $n_1=3$, $n_2=21$, $p<0.001$). The neighbour-joining tree based on Nei's D also mirrored the early reintroduction history of Alpine ibex. Thus, very similar results were obtained with (Nei's D) and without (STRUCTURE) a priori knowledge of population identity. We found three distinct clades, representing the captive-founded populations and populations that received individuals from those (Figure 3). Populations not belonging to one of these three clades are the ancestral population and three of the mixed-founded populations (ab, sm, we). The other mixed-founded populations (ap, gh, go and pg) belonged to the clade of the source of their wild-founded populations in the neighbour-joining tree. Similarly there are wild-founded populations descending from more than one source, but they belonged clearly to one of their sources only (e.g. fl and av population) according to the genetic distance analysis and cluster analysis.

Discussion

Genetic variation and the influence of serial bottlenecks

We found low levels of microsatellite genetic variation among 39 Alpine ibex populations, similar to those previously found in seven Alpine ibex populations in France, Switzerland and Italy (Maudet *et al.* 2002). Only species that have very small effective population sizes or that have experienced serial founder events have similarly low levels of genetic variation (Goodman *et al.* 2001; Garner *et al.* 2005; Jamieson & Grueber 2006). Related species, e.g. the Iberian Ibex (Amills *et al.* 2004) and goat breeds (Canon *et al.* 2006) have 56% and 68% higher variation of He and on average 0.8 and 8.5 more alleles per marker respectively, though these species also experienced bottlenecks. The microsatellite loci we used were not developed in Alpine ibex and He and Na might thus be downward biased in our study (Ellegren *et al.* 1995). However, if we compare the eight markers derived from cattle and sheep that are common to the goat and our Alpine ibex study, goat breeds still have more than fourfold higher Na and nearly twice as high He. Thus, the low genetic variability in Alpine ibex does not seem to be a consequence of ascertainment bias alone and is concordant with the loss of genetic variation expected from founder events (Nei *et al.* 1975).

Although we found lower genetic variation in each Swiss population than in the ancestral population, all Swiss populations combined had the same He and more alleles than the ancestral gp population. While the 19-fold difference in sample size might explain the higher Na in the combined Swiss sample, He is not affected in the same way by sample size. Thus, the number of animals (~100) brought to Swiss zoos was sufficient to bring most of the genetic variation of the ancestral population to Switzerland as expected from theory (Denniston 1978). However, genetic variation was subsequently split up between Swiss populations due to founder effects and a lack of migration between many of the populations. This corresponds to the expectation from theory that population structure can transfer genetic variation within populations to genetic variation among populations (Gaggiotti & Couvet 2004). This pattern that genetic variation within a

source population is split up into variation between descended populations might be common in reintroduced populations (O`Ryan *et al.* 1998).

Most reintroduced populations are exposed to single or serial founder events because such reintroductions are usually done with few founder individuals. Where ibex populations experienced serial bottlenecks, their effects on Na diminished, i.e. the first bottleneck reduced the number of alleles more than subsequent bottlenecks. This is predicted from theory given that low frequency alleles are preferentially lost during a bottleneck (Wright 1931; Maruyama & Fuerst 1985; Allendorf 1986). When only alleles at higher frequencies remain in a population after a founder event, fewer founder individuals are necessary to preserve most of the alleles present in a source population. Evidence for substantial genetic drift was found even in those situations where no additional loss of Na was observed. While additional bottlenecks did not proportionally reduce the number of alleles, He was lost at the same rate in subsequent bottlenecks, leading to situations where genetic drift but no additional loss of alleles is observed. Therefore, while Na is a more sensitive measure of a single bottleneck than He, serial bottlenecks continue to affect He and an absence of additional declines in Na does not imply that additional bottlenecks have no effects (Taylor & Jamieson 2008). The cumulative effect of loss of He per bottleneck is also reported for the reintroduced moose in Canada (Broders *et al.* 1999). Our findings for neutral loci may as well apply to selected loci given the small population sizes during reintroductions because at low population size random processes may overwhelm selection.

Genetic Structure and the influence of reintroduction history

A strong footprint of the reintroduction history was evident with and without a priori knowledge of population identity. A Bayesian cluster analysis revealed three clusters representing the three captive-founded populations. Thus, the number of clusters did not represent the number of populations, but instead the early reintroduction history (Figure 2). Likewise, the three captive-founded populations and their descendants formed distinct clades in the neighbour-joining tree (Figure

3). Scribner and Stuwe (1994) found the same topology among three populations that were common to their allozyme study of Alpine ibex. Strong genetic drift in the captive-founded populations is likely responsible for this pattern. High impact of the captive-founded populations was also seen in the distribution of genetic variation. Genetic variation among populations was more than two thirds influenced by reintroduction history, indicating that a substantial proportion of genetic variation was partitioned between the historical groups.

Strong genetic drift in the captive-founded populations and less drift in the wild-founded populations might have several causes. First, genetic distances between populations can be increased rapidly if one is experiencing a bottleneck (Chakraborty & Nei 1977; Hedrick 1999). Thus two bottlenecks result in higher genetic drift than only one bottleneck, which is in line with the observation that He was lost with each founder event. The captive-founded populations experienced two founder events compared to the source used in the study (gp), while many of the wild-founded populations experienced only one bottleneck compared to their source populations. Second, founding individuals originating from captivity might have been more related than founding individuals from natural populations. In captivity population sizes are generally low and thus animals are more related. The release of related individuals might have reduced the effective founder size in the captive-founded group compared to the wild-founded group and consequently enhanced genetic drift (Whitlock & McCauley 1990), even if the same number of individuals were released. Third, assuming a generation time of 8 years (Stuwe & Grodinsky 1987), captive-founded populations had on average 4.5 generations more time to diverge genetically than wild-founded populations.

As expected, the ancestral and some mixed-founded populations did not group with one of the three captive-founded clades in the neighbour-joining tree (Figure 2). However, four admixed populations (ap, fl, gh and go) belong clearly to one of the captive-founded clades and clusters. Presumably, in these populations founders of one source contributed much more than any others to today's gene pool. This interpretation is supported by observations for the fl and gh population, where it is known that only few animals of one of the sources survived long

enough to reproduce (Tschirky 2004). This reiterates that the outcome of reintroduced populations depends greatly on the survival and reproduction of founder individuals. While one can plan who to release one can rarely prevent these (random) effects.

Conclusion

Our assessment of genetic structure and differentiation among ibex populations provides important information for conservation and management. Despite the crudeness of the available measures of the reintroduction history, the strongest pattern visible in today's genetic structure of Alpine ibex is a consequence of their reintroduction history and not of ibex life history. Translocations between populations belonging to different historical groups would increase standing genetic diversity within populations. The same might be eventually achieved by waiting until gene flow is established among the populations but the life history of ibex combined with the topography of their habitat makes this a very slow process.

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Table 1: Reintroduction history and genetic parameters for 42 Alpine ibex populations.

population	pop. code	found. type	rel. Ind.	hist. groups	bottle	N	He	Ho	Na	%AF <0.05
Adula-Vial	av	wild	46	none	NA	37	0.40	0.38	2.54	11.7
Albris	al	captive	42	al	1 to 2	61	0.41	0.42	2.54	9.6
Aletsch-Bietschhorn	ab	mixed	57	none	NA	43	0.47	0.46	2.92	10.2
Alpstein	ap	mixed	12	none	NA	30	0.35	0.34	2.27	9.5
Arolla	ar	wild	96	pl	3 to 4	36	0.43	0.44	2.70	10.0
Bire-Oeschinen	bo	wild	13	br	3 to 4	18	0.41	0.43	2.51	7.5
Brienzer-Rothorn	br	captive	18	br	1 to 2	39	0.46	0.46	2.57	5.3
Calanda	ca	wild	36	al	3 to 4	31	0.37	0.37	2.27	7.1
Cape au Moine	cm	wild	NA	none	NA	49	0.42	0.42	2.65	10.2
Churfirsten	ch	wild	27	none	NA	24	0.41	0.42	2.57	6.3
Crap da Flem	cf	wild	39	al	3 to 4	27	0.40	0.39	2.32	2.3
Dents du Midi	dm	wild	21	pl	3 to 4	23	0.44	0.42	2.60	8.3
Ferret	fe	wild	47	none	NA	19	0.41	0.39	2.65	9.2
Fluebrig	fl	wild	21	none	NA	32	0.39	0.39	2.49	7.6
Flueela	fu	wild	42	al	3 to 4	21	0.36	0.35	2.30	4.7
Foostock	fo	wild	12	none	NA	27	0.38	0.41	2.35	8.0
Gornergrat	go	mixed	10	none	NA	23	0.40	0.38	2.60	13.5
Gran Paradiso	gp	ancestral	NA	none	NA	56	0.45	0.42	3.08	17.5
Graue Hoerner	gh	mixed	55	none	NA	47	0.40	0.41	2.51	8.6
Gross Lohner	gl	wild	33	none	NA	22	0.46	0.44	2.92	17.6
Hochwang	hw	wild	40	al	3 to 4	28	0.38	0.39	2.32	2.3
Julier Nord	jn	wild	109	al	3 to 4	19	0.39	0.39	2.30	4.7
Julier Sued	js	wild	41	al	3 to 4	23	0.39	0.37	2.41	11.2
Justistal	ju	wild	25	br	3 to 4	19	0.41	0.43	2.38	5.7
Macun	ma	wild	53	al	3 to 4	22	0.38	0.38	2.43	6.7
Mischabel	mi	wild	25	pl	3 to 4	33	0.40	0.39	2.54	5.3
Muveran	mu	wild	58	pl	3 to 4	27	0.38	0.39	2.65	8.2
Nufenen	nu	wild	33	none	NA	19	0.38	0.39	2.60	8.3
Oberbauenstock	ob	wild	23	al	3 to 4	30	0.34	0.34	2.22	11.0
Pierreuse-Gummfluh	pg	mixed	14	none	NA	41	0.47	0.49	2.84	12.4
Pilatus	pi	wild	17	al	3 to 4	17	0.36	0.40	2.30	9.4
Pleureur	pl	captive*	78	pl	1 to 2*	23	0.42	0.43	2.73	12.9
Rheinwald	rh	wild	29	al	3 to 4	35	0.38	0.38	2.49	14.1
Rothorn-Weissfluh	rw	wild	77	al	3 to 4	29	0.42	0.42	2.38	5.7
Schwarzmoench	sm	mixed	26	none	NA	32	0.45	0.44	2.68	10.1
Tanay	ty	wild	9	pl	3 to 4	25	0.40	0.39	2.46	3.3
Umbrail	um	wild	59	al	3 to 4	29	0.41	0.40	2.51	5.4
Val Bever	vb	wild	137	al	3 to 4	32	0.40	0.38	2.49	8.7
Weisshorn	wh	wild	24	pl	3 to 4	25	0.36	0.37	2.57	16.8
Weissmies	wm	wild	31	none	NA	49	0.39	0.39	2.70	9.0
Wetterhorn	we	mixed	21	none	NA	19	0.42	0.43	2.41	1.1
Wittenberg	wb	wild	21	none	NA	21	0.44	0.44	2.73	10.9
Mean						30	0.41	0.40	2.54	8.8

pop.code: abbreviation for each population; found.type: index of founding type (see text); rel.Ind.: number of released individuals; hist. groups: index of historical groups (see text); bottle: number of experienced bottlenecks; N: number of samples; He: expected heterozygosity; Ho: observed heterozygosity; Na: number of alleles per locus; %AF<0.05: percent alleles below 0.05 frequency

* pl received animals from captive and wild Swiss populations, but the two Swiss populations were founded by pl itself and thus pl was defined as captive-founded.

Table 2: Analysis of covariance for the effect of serial bottlenecks on He, Na and rare alleles (frequency < 0.05).

	1 - 2 founder events mean±se	3 - 4 founder events mean±se	p founder event	p released individuals
N	3	21		
He loss	-0.020 ± 0.015	-0.027 ± 0.006	0.681	0.316
Na loss	-0.647 ± 0.071	0.049 ± 0.037	<0.001***	0.613
AF<0.05 loss	-0.297± 0.062	-0.078 ± 0.022	0.003**	0.900

N: sample size of the class; p founder event: p value for the experienced founder events; p released individuals: p value for ln of released founder individuals.

***P<0.001; **P<0.01; *P<0.05

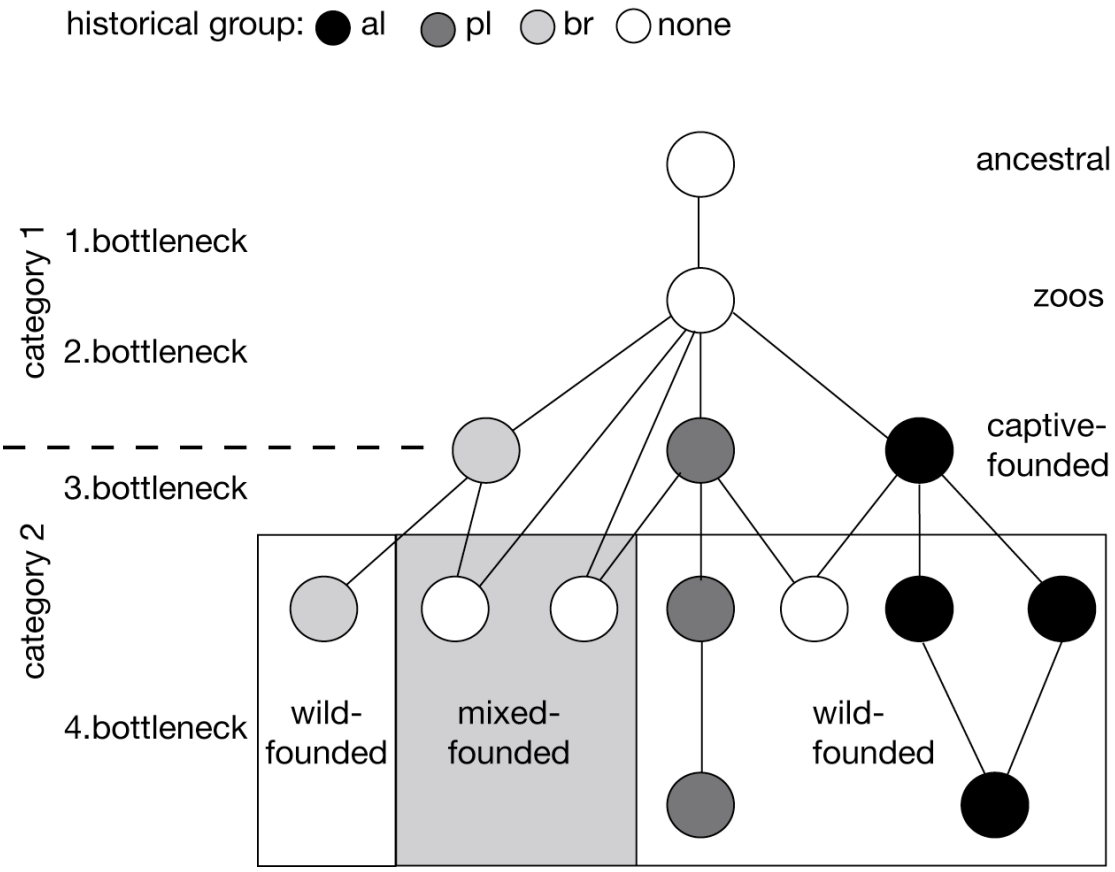

Figure 1: Schematic illustration of the Alpine ibex reintroduction history

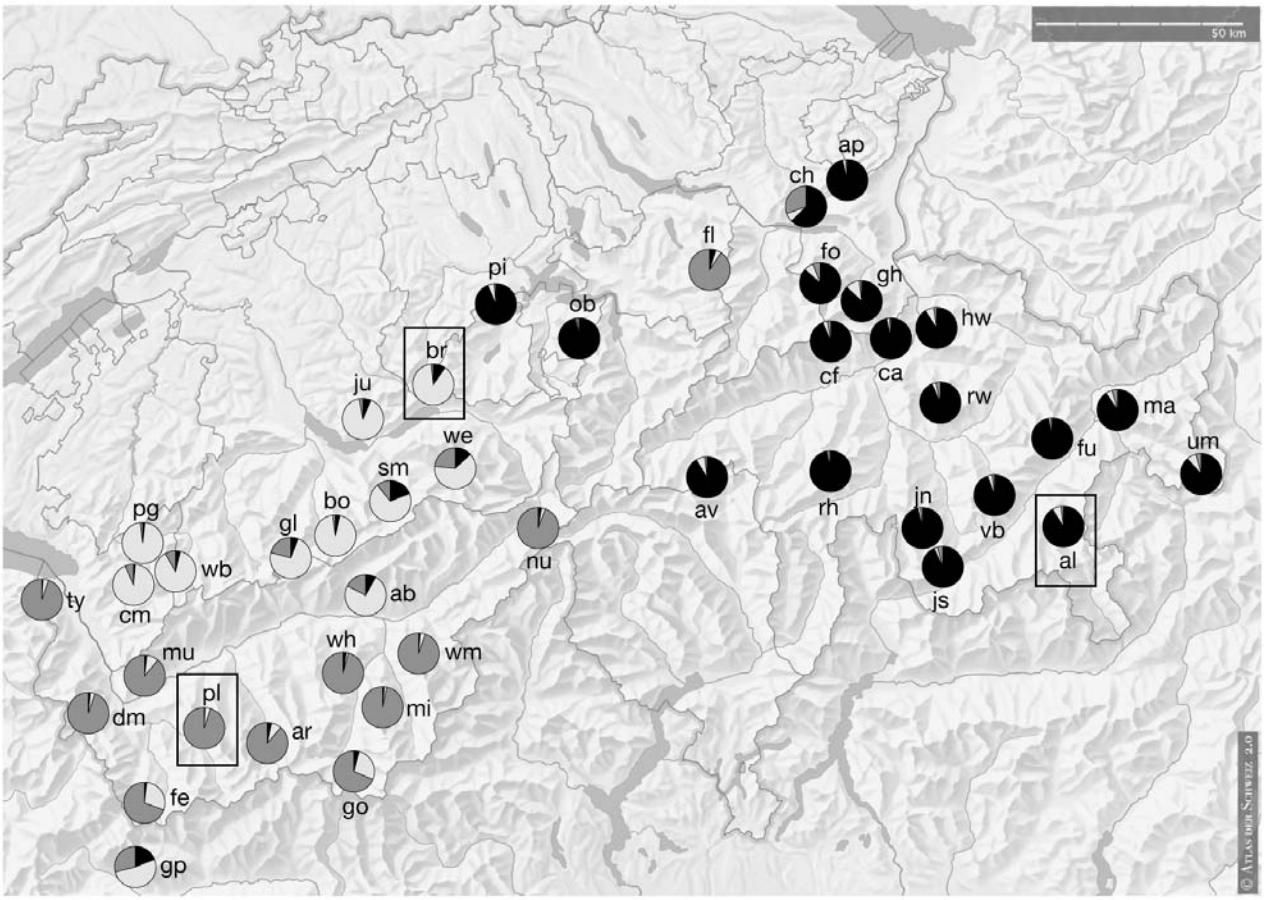

Figure 2: Cluster analysis (STRUCTURE) for K=3. The segments show the proportion of membership of the populations to three clusters. Population names are as in Table 1. The boxes indicate the three captive-founded populations. The underlying map is taken from the software Atlas of Switzerland 2.0 (2004).

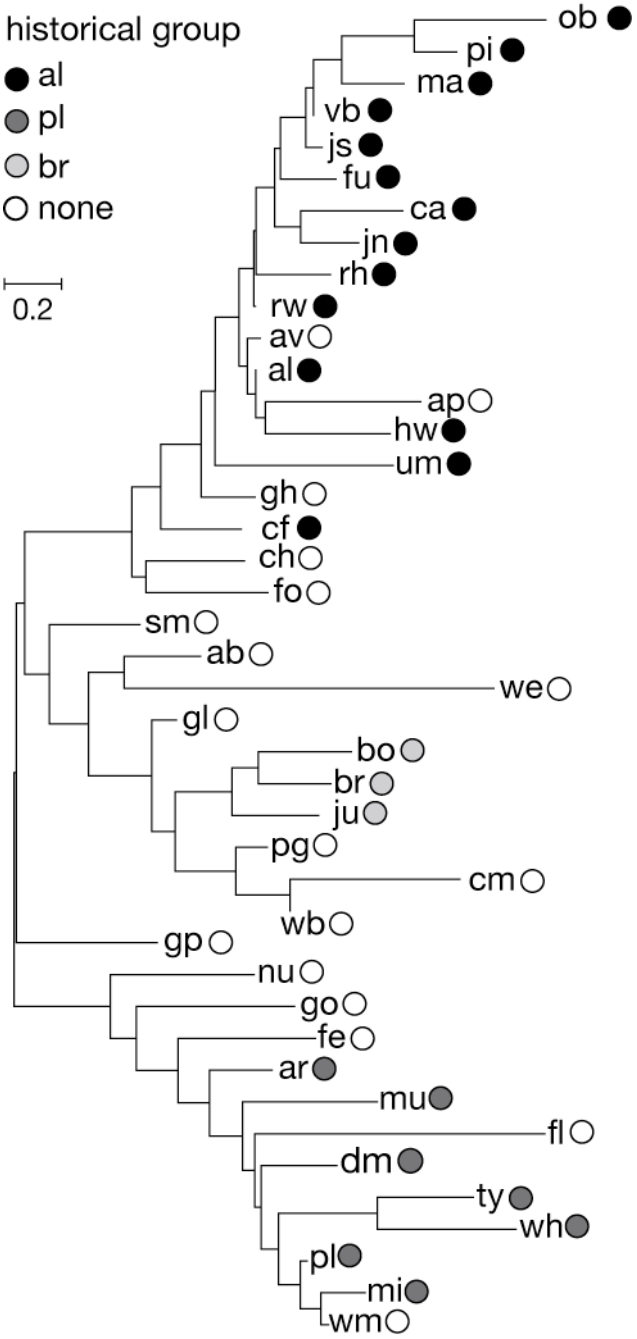

Figure 3: Unrooted neighbour-joining tree using Nei's D as genetic distance.

Appendix 1: List of microsatellites used in this study

FA	PCR	Ann. Temp.	locus	Drop-out rate	false allele rate	Chr[species]	GB acc.nr.	source
1	A	57	OarVH34	0.000	0.000	5 [Capra hircus]	L12559	Pierson et al. (1993)
1	A	57	BM4208[c]	0.009	0.000	9 [Bos taurus]	G18509	Bishop et al. (1994)
1	A	57	MCM152	0.000	0.000	13 [Bos taurus]	L39825	Davies et al. (1995)
1	A	57	BM302	0.000	0.000	14 [Bos taurus]	G18774	Bishop et al. (1994)
1	A	57	MAF209	0.000	0.000	17 [Ovis aries]	M80358	Buchanan et al. (1992a)
1	D	57	TGLA126	0.000	0.000	20 [Bos taurus]	191460	Georges et al. (1992)
1	D	57	INRABERN172	0.000	0.000	26 [Bos taurus]	NA	Vaiman et al. (1996)
1	D	57	INRABERN185	0.000	0.006	NA [Bos taurus]	NA	Kappes et al. (1997)
1	G	55	SR-CRSP23	0.000	0.000	NA [Capra hircus]	NA	Yeh et al. (1997)
2	B	59	ILSTS30	0.005	0.008	2 [Bos taurus]	L37212	Kemp et al. (1995)
2	B	59	OarFCB20	0.000	0.000	2 [Bos taurus]	L20004	Buchanan et al. (1994)
2	B	59	SR-CRSP01	0.038	0.000	NA [Capra hircus]	NA	Arevalo et al. (1994)
2	B	59	BM1225	0.007	0.007	20 [Bos taurus]	G18419	Bishop et al. (1994)
2	B	59	IDVGA30	0.000	0.000	21 [Bos taurus]	X85049	Mezzelani et al. (1995)
2	B	59	TGLA122	0.000	0.000	21 [Bos taurus]	NA	Georges et al. (1992)
2	B	59	JMP29	0.025	0.000	24 [Ovis aries]	U30893	Penty et al. (1995)
2	B	59	SR-CRSP24	0.000	0.000	NA [Capra hircus]	NA	Yeh et al. (1997)
2	H	56	MCM73	0.000	0.000	4 [Ovis aries]	L34285	Hulme et al. (1994)
2	H	56	TGLA73	0.012	0.000	9 [Bos taurus]	NA	Barendse et al. (1994)
2	H	56	SR-CRSP08	0.000	0.000	NA [Capra hircus]	NA	Bhebhe et al. (1994)
3	C	62	ETH10[b]	0.019	0.000	5 [Capra hircus]	Z22739	Solinas et al. (1993)
3	C	62	BM415	0.000	0.000	6 [Bos taurus]	G18413	Bishop et al. (1994)
3	C	62	OarFCB48	0.000	0.000	17 [Capra hircus]	M82875	Buchanan et al. (1994)
3	C	62	BM4505	0.074	0.000	26 [Bos taurus]	G18511	Bishop et al. (1994)
3	C	62	MAF36	0.000	0.000	26 [Bos taurus]	M80519	Swarbrick et al. (1991)
3	C	62	OarKP6[a]	0.000	0.000	3 [Ovis aries]	AF223411	Paterson et al. (2000)
3	C	62	SR-CRSP25	0.025	0.000	NA [Capra hircus]	NA	Yeh et al. (1997)
4	E	59	ILSTS29	0.000	0.005	3 [Capra hircus]	L37252	Kemp et al. (1995)
4	E	59	CSSM47	0.071	0.000	8 [Bos taurus]	U03821	Moore et al. (1994)
4	E	59	MILSTS076	0.000	0.000	9 [Bos taurus]	9982	Kemp et al. (1995)
4	E	59	OARFCB193	0.000	0.000	19 [Capra hircus]	L01533	Buchanan et al. (1993)
5	K	55	SR-CRSP07[d]	0.000	0.000	NA [Capra hircus]	NA	Bhebhe et al. (1994)
5	K	55	TGLA10	0.000	0.000	8 [Bos taurus]	NA	Barendse et al. (1994)
5	K	55	URB058	0.000	0.000	13 [Bos taurus]	U21788	Ma et al. (1996)
5	K	55	BM1258[a]	0.000	0.000	23 [Bos taurus]	G18385	Bishop et al. (1994)
5	K	55	BM1818[a]	0.026	0.000	23 [Bos taurus]	G18391	Bishop et al. (1994)
5	K	55	INRABERN175	0.010	0.000	25 [Bos taurus]	NA	Vaiman et al. (1996)
5	K	55	HAUT27	0.020	0.000	26 [Bos taurus]	NA	Thieven et al. (1997)
6	F	54	BM2113	0.000	0.000	2 [Bos taurus]	M97162	Bishop et al. (1994)
6	F	54	SR-CRSP09	0.000	0.000	12 [Capra hircus]	NA	Bhebhe et al. (1994)
6	I	62	MAF70	0.000	0.000	4 [Bos taurus]	M77199	Buchanan et al. (1992b)
6	I	62	OarHH35	0.000	0.000	4 [Ovis aries]	L12554	Pierson et al. (1993)
6	I	62	OarHH62[c]	0.011	0.000	16 [Ovis aries]	L13872	Ede et al. (1994)
6	I	62	OarAE54	0.071	0.000	25 [Ovis aries]	L11048	Penty et al. (1993)

FA: fragment analysis run; PCR: multiplex reaction for PCR; Ann.temp: annealing temperature in PCR reaction; locus: name of microsatellite; Chr.[species]: chromosomal location of the microsatellite for the given species; GB access.nr: Genbank accession number; reference: reference of the microsatellite

a According to the bovine linkage map (NCBI map viewer) and Paterson & Crawford (2000) microsatellites are linked to genes under selection.

b ETH10 is possibly under selection in Alpine ibex (von Hardenberg 2007).

c possibly under selection by FDIST2 analysis.

d significant excess of homozygotes.

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2 GENETIC VARIATION DEPENDS MORE ON ADMIXTURE THAN NUMBER OF FOUNDERS IN REINTRODUCED ALPINE IBEX POPULATIONS

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Submitted to Conservation Biology

Abstract

In recent years many populations have been reintroduced, however, the success of reintroductions is highly variable. Genetic variation may influence the success and therefore, along with other considerations reintroductions should aim to preserve much of the genetic variation. Genetic variation can be increased by increasing the founder group size or the admixture, however, up to date there is no clear understanding of the relative importance of the two. Here we address this issue by using detailed information about the reintroduction history of 40 Alpine ibex populations together with genetic data from neutral markers. Additionally we estimated the number of genetic founders with a coalescent-based method to get an estimate of the survival of the released founders and explore its effects on genetic diversity compared to the number of physically released founders. Genetic founders were better predictors of the present genetic variation than released founders indicating that differential survival of founders can substantially affect the genetic variation of reintroduced populations. The degree of admixture in the founder group had about twice as much impact on genetic variation than the founder group size. Thus, to maintain genetic variation of reintroduced populations that experienced several founder events or became recently isolated, releasing animals from different sources might be more important than releasing many animals from a single source.

Introduction

Human activities have driven many species to extirpation and extinction within the last two centuries. Some of these species have been reintroduced to their former habitat, often after breeding in captivity (Stanley & Mark 1989; Jones *et al.* 1995). The success of such reintroductions has been highly variable. Two post-hoc assessments of reintroduction programs revealed that an important parameter increasing reintroduction success was the number of released animals (Wolf *et al.* 1996; Breitenmoser *et al.* 2001). Environmental, demographic and genetic factors may explain this positive effect of the number of founder individuals. Genetic factors can influence population persistence and hence the success of reintroduced populations in two ways. Firstly, when only few individuals are released, after only a handful of generations their descendants will be fairly closely related, leading to inbreeding and potentially to inbreeding depression and reduced fitness (Keller & Waller 2002). Secondly, founder effects and, as long as the population size remains low, genetic drift will lead to loss of genetic variation (Crow & Kimura 1970; Nei *et al.* 1975). This reduces the genetic variation upon which natural selection can act and thus the ability to adapt to changing environments (James 1970; Allendorf & Luikart 2007). Therefore, along with other considerations, reintroduction programs should try to reduce inbreeding in the newly founded populations and preserve as much as possible of the pre-existing genetic variation.

Managers can minimize the loss of genetic variation during reintroduction programs by increasing the number of founder individuals (Nei *et al.* 1975; Maruyama & Fuerst 1985) or by admixture between animals from different sources, provided that several sources exist and if they are genetically differentiated (Moritz 1999; Maudet *et al.* 2002; Dlugosch & Parker 2008). Admixture is a potentially powerful way to increase genetic variation and may be one of the factors contributing to the success of invasive species: several studies suggest that admixture following multiple introductions increase the establishment rate of various species (e.g. Kolar & Lodge 2001; Hanfling 2007). Multiple introductions from different sources have been shown to increase

genetic variation in invasive plants (Ellstrand & Schierenbeck 2000; Genton *et al.* 2005; Marrs *et al.* 2008) and in invasive animals (Kolbe *et al.* 2004, 2007) above the level of each individual source population. While in invasive species high diversity is undesirable because it may enable these species to better respond to selection pressures and adapt to their new environment (Kolar & Lodge 2001; Dlugosch & Parker 2008), this ability to evolve is desirable in reintroduced populations. Although several reintroductions with mixed source populations are known (DeYoung *et al.* 2003; Latch & Rhodes 2005; Taylor & Jamieson 2008), we currently lack a clear understanding of the relative importance of admixture versus founder population size for genetic variation in reintroduced populations. Such an understanding could inform the decision making process in future reintroduction programs: with limited financial and personnel resources one has to decide if it is better to release more animals from one source or fewer animals from different sources.

Here we investigate the relative impact of admixture (i.e. the number and relative contribution of source populations) and founder population size on genetic variation at neutral genetic markers in the reintroduced Alpine ibex (*Capra ibex ibex).* Alpine ibex populations are well suited to investigate this question for three reasons. First, as is typical for many reintroduced species, they experienced several founder events and thus genetic variation among Alpine ibex populations varies considerably. Populations differ by as much as 29% for expected heterozygosity and 37% for number of alleles (Chapter 1). Second, the reintroduced populations share one common ancestral population and, due to the life history of Alpine ibex, no or very limited gene flow occurs between populations (Nievergelt 1966). Thus, theoretical predictions are straightforward and allow comparisons with empirical observations that, for most of the populations, are not confounded by gene flow. Third, the reintroduction histories of the populations are documented in unusual detail and vary with respect to the number of founders and the degree of admixture.

Methods

Alpine ibex history

Grodinsky & Stuwe (1987), Stuwe & Nievergelt (1991) and Chapter 1 give a detailed account of the history of Alpine ibex and we only summarize it briefly here. Following extirpation Alpine ibex were successfully bred in captivity in Switzerland using individuals from the sole surviving population in northern Italy, the Gran Paradiso (gp) population. These captive-bred Alpine ibex served as sources for the establishment of the first wild populations and, after an increase in population size, animals from mainly three of these wild populations were used for further reintroductions. Individuals in the founder groups of the Swiss populations came from one to four source populations. To investigate the effects of admixture and founder numbers on genetic diversity we studied 40 reintroduced Alpine ibex populations in Switzerland for which the founding period, the origin, and the number of founder individuals were well documented (Table 1). Details of the population definition and reintroduction histories of the studied populations are given in Chapter 1.

We quantified admixture of the founder group with an admixture index that was calculated as the Shannon Wiener index (Shannon 1948) of the number of founder individuals originating from different source populations, i.e., *admixture index* $= -\sum_{i=1}^{n} Pi \ln Pi$ where Pi is the proportion of founder individuals that originated from the source population i and n is the number of sources. Thus admix is high if several sources contributed founder individuals and if their contribution was relatively equal. Populations that descended from one, single wild population were not strongly differentiated from their source (Chapter 1). Thus, founder individuals from such populations are not expected to differ in their effect on the genetic variation of the recipient population than individuals from their source. Therefore, we categorized them as the same source for the calculation of the admixture index. Thus, the possible source populations were: the ancestral gp population, four zoos (dh, ih, pp, se) and six reintroduced wild populations (ab, al, ap, br, gh and pl).

Genetic data

Genetic data were obtained for these 40 populations using 37 microsatellite loci. Details of the loci used and the laboratory conditions are given in Chapter 1. There we found that all 40 populations were in Hardy-Weinberg equilibrium across all loci, suggesting no hidden substructure in the defined populations.

We calculated expected heterozygosity and number of alleles for each population in ARLEQUIN (Excoffier *et al.* 2005) to quantify genetic variation. We used both measures because H_e and Na have been shown to behave differently during a bottleneck: both measures depend on the bottleneck size (Wright 1951), but He is more sensitive to bottleneck duration and thus to the population growth rate after the bottleneck while Na is more sensitive to bottleneck size (Nei *et al.* 1975; Denniston 1978; chapter 1). We estimated Na using the absolute number of alleles and not allelic richness because the proportions of the population sampled varied tremendously. Thus, our results will be less biased without standardization (Chapter 1).

Genetic founders

Often not all individuals of a founder group will survive long enough to contribute genetically to the next generation (Bar-David *et al.* 2005; Jule *et al.* 2008) and the proportion of surviving individuals might vary among populations. The number of released individuals will then be an inaccurate and imprecise estimator of the founder group size of a population. To get a better estimate of the number of founder individuals we used NFCONE, a program that implements a maximum likelihood method based on the coalescent to estimate the number of founding chromosomes (Anderson & Slatkin 2007). The method uses contemporary genetic samples of both the founded and the source population and allows for genetic drift in the founded but not the source population (Anderson & Slatkin 2007). Therefore the estimated number of founder chromosomes is the number of lineages ancestral to the present samples in addition to the number of lineages that were lost due to genetic drift. In the following, we will call the estimate of the

number of founder chromosomes divided by two the number of genetic founders, and the number of physically released individuals the number of released founders.

To account for the genetic drift in the founded population, assumptions about its population growth rate have to be made. Because an overestimate of the intrinsic rate of increase (r) creates less bias than an underestimate (Anderson & Slatkin 2007), we assumed an r of 0.25 for all ibex populations, a number that is near the maximum reported for this species (Toigo *et al.* 1996; Loison *et al.* 2002), a generation time of eight years (Stuwe & Grodinsky 1987), and a carrying capacity identical to the maximum census count of each population (obtained from hunting authorities and agencies; Table 2). The unknown true r is probably not much lower because the populations under investigation grew rapidly after reintroduction, but a lower true r will result in us underestimating the number of founding chromosomes. Since we were primarily interested in relative rather than absolute numbers, such biases are not particularly serious as long as they affect all populations similarly. Note furthermore that Anderson and Slatkin's (2007) method assumes a single source population. Therefore, we were only able to estimate the number of founding chromosomes for the 22 populations that originated from a single source. Thus, admixture index is zero in all these populations.

Data Analysis

The effect of the number of founders and degree of admixture on genetic variation was investigated with several models with either He or Na as the dependent variable. The first set of models had the natural logarithm of the number of released founders, founder year and admixture index for each population as independent variables. We included founder year because the time since founding might have influenced the genetic diversity, as the longer ago a population has been established, the more time has passed for genetic drift to occur. We log-transformed the number of founder individuals because genetic diversity is expected to follow an asymptotic relationship with number of released

individuals (Denniston 1978). The second set of models used the number of genetic founders instead of the released founders to investigate the effect of unequal founder survival (or reproduction). However, we only had estimates of the number of genetic founders for 22 populations. To be able to use all 40 populations and to compare the resulting models, we combined the two variables, number of genetic and released founders in a new variable called combined founders. To correct for possible differences in the mean and variance between number of genetic and released founders we used a z score standardization for both variables after log-transformation. We then replaced the number of released founders with the number of genetic founders for the 22 populations for which the genetic estimator could be calculated and kept the number of released founders for the remaining populations. Models were either multiple regressions or linear mixed effect models with an additional random effect term accounting for the source of the population. Genetic diversity of the source populations differs (Chapter 1) and therefore the identity of the source populations might affect the level of genetic diversity of the founded populations. Hence, we included source (Table 1) as a random effect. Model selection was only done for the interaction terms and the random effect. Thus all models had the admixture index, founder year and number of released or combined founders as explanatory variables and differed only in the interaction terms or random effect. All variables were standardized to be able to compare the relative impact of the different variables.

We used AICc, the Akaike Information Criterion (AIC) corrected for small sample size for model selection, with lower AICc scores indicating better support for the model (Burnham & Anderson 2002). Models with a difference in AICc values of less than two were considered to be equally likely descriptions of the causes of variation (Burnham & Anderson 2002). For the three models with lowest AICc for He and Na (Table 3) we calculated the significance of the variables by the F-ratio test for the models without random effects and Chi square test for the models with random effects. All statistical analyses were carried out with the R package, version 2.6.1 (R Development Core Team 2006).

Results

Reintroduction history and number of genetic founders

Admixture index, founder number and founder year, the three variables hypothesized to affect genetic diversity, varied across populations. All ibex populations were founded within the last 100 years, but the time for possible loss of genetic diversity differed between 3 and 12 generations. The admixture index was zero for the 22 populations that were founded from only one population and ranged between 0.3 and 1.3 for the remaining populations. The mean number of released individuals was 39 with a range between 9 and 137 (Table 1). The estimates of the number of genetic founders were lower (mean: 21, range: 7 – 78) than the number of released founders for all but four populations (bo, mi, ty, fo; Table 2). Excluding these four populations, between 13-100% (mean = 45%) of the released individuals can explain the genetic variation we see today in the populations. The 95% upper confidence limits included the number of released individuals in ten populations suggesting no substantial difference between number of released and genetic founders in these populations (Figure 1). The correlation between the number of genetic and released founders was r=0.59, thus variation in the number of released founders explained 35% of the variation in the number of genetic founders.

Influence of founder number and admixture on genetic variation

The absolute magnitude of the correlation coefficients between the three explanatory variables in the models, admixture index, founder number and founder year, were below 0.45 suggesting that multicollinearity was not a serious problem.

Expected heterozygosity of a population was best described by a model with the combined founders instead of number of released founders, and without random effects of the source population. This model accounted for 29.3% of the variation in He and only admixture index was significant. There was a trend

(p=0.13) for the combined founders to affect expected heterozygosity, but this effect was less than half as strong as the admixture index (Table 3). The second best model for He, only slightly less plausible than the first, additionally included the source as random effects. In that model, both admixture index and combined founders had a significant influence on He with similar coefficients as in the first model. Founder year did not influence the expected heterozygosity of the populations. The third model had the same variables as the first model except that combined founders were replaced with number of released founders. This model was less supported by the AICc as it differed by 2 AICc points from the best model. It explained only 25.7% of the variation and of the variables only admixture index was significant. Neither founder year nor number of released founders had an effect.

In contrast to He, source as random effects was included in the three best models explaining variation in Na in the populations. The best model was one containing combined founders, but the second and third best model included number of released founders (Table 3). The best model differed by more than 2 AICc points from the other models, suggesting that the number of genetic founders is a better predictor of Na in the present-day populations. Admixture index and founder number, either combined or released, were significant in all three models, but founder year did not influence Na. Admixture index had about twice as much influence on Na as the number of founder individuals. One model included the interaction between founder year and number of released founders. However, the model with this interaction term differed barely in AICc from a model without the interaction.

Discussion

Genetic founders

The number of genetic founders was in general less than the number of released founders suggesting that only a fraction of the released individuals was needed to explain the present genetic variation. In other words, present-day populations

have lower genetic variation than what one would expect given the number of animals released. The differences between the number of released and genetic founders represents founder animals that did not survive nor reproduced or founder individuals who were close relatives such that fewer animals are needed to explain today's genetic variation. Since we had to make assumptions about the demographic history of the populations, we cannot exclude the possibility that the estimates of the number of genetic founders might be biased (Anderson & Slatkin 2007). However, the estimated number of genetic founders and the actually released founders were correlated, suggesting that our data contain some information about the number of genetic founders in the populations. We were not particularly interested in the absolute numbers of genetic founders, but rather in the relative comparison among the populations. Thus, bias that affects all populations in the same manner is a minor problem.

Gene flow might be responsible for the high number of genetic founders in relation to released individuals in four populations (bo, mi, ty, fo). If gene flow occurs, the assumption of an isolated population as required by the model, is violated. From one of these populations (fo) we know that gene flow is possible as it was founded by natural dispersal and gene flow likely continues (Tschirky 2004).

Influence of founder number and admixture on genetic variation

The admixture index had twice as much effect on genetic variation than founder group size, if founder group size influenced genetic variation at all. The more even the proportion of animals from different source populations and the higher the number of source populations was, the higher was the present-day genetic variation. Our results are in accordance with three observations: (1) Populations that were restored from different sources and admixed due to population expansion lost less genetic variation (DeYoung *et al.* 2003; Latch & Rhodes 2005). (2) Few immigrants can restore the genetic variation that was lost during a population bottleneck (Keller *et al.* 2001). (3) There is a positive relationship between genetic variation and number of sources in introduced populations

(Ellstrand & Schierenbeck 2000; Kolbe *et al.* 2004; Latch & Rhodes 2005; Dlugosch & Parker 2008; Kolbe *et al.* 2008).

The number of genetic founders included in the combined founder variable rather than the number of released founders entered the most plausible models for both He and Na. This emphasizes that differential survival of founders and/or relatedness among founders can significantly affect the genetic variation of reintroduced populations, suggesting that monitoring founder survival and relatedness might be an important aspect of management.

The best fitting models differed between He and Na with respect to the random effects. While the source population as random effects was important for fitting the data for Na, it did not improve the fit for He. This corresponds to the theoretical expectation that the loss in Na is more influenced by the level of genetic variation of the source population than He (Kimura 1955). Furthermore Na is a more sensitive measure of the bottleneck effect than He (Denniston 1978). The level of Na is mainly dependent on the bottleneck size, while He is additionally influenced by the growth of the populations after the bottleneck (Nei *et al.* 1975; Allendorf 1986; Leberg 1992). Accordingly, various studies have shown bottleneck effects in Na but not in He (Fitzsimmons *et al.* 1995; Williams *et al.* 2000; Clegg *et al.* 2002; Dlugosch & Parker 2008). The higher sensitivity of Na was also found in our study, where the number of released founders did not influence He, but influenced Na significantly. Depleted source populations and high relatedness of the founder group might be other factors for the unexpected absence of an effect of the released founder group size on He. From source populations with low genetic diversity less founder animals are needed to transfer the genetic diversity into the new population, but the asymptotic level is higher for Na than for He (Denniston 1978, p. 283). Alpine ibex exhibit low genetic diversity (Chapter 1) and therefore released founder numbers might have been above the asymptotic level for He but not for Na. Founder year did not have a direct effect on genetic variation in either model suggesting that the founding times of the populations were too similar to find an effect of the different lengths of genetic drift.

Conclusion and Management implications

In reintroduction programs, some loss of genetic variation often cannot be prevented. However, reintroduction programs should endeavor to loose as little variation and create as little inbreeding as possible. Using post-hoc genetic analyses of a successful reintroduction of Alpine ibex, we showed that admixture through the representation of different source populations in the founder group can increase genetic diversity in the reintroduced population. Similarly (Kolbe *et al.* 2007; Kolbe *et al.* 2008) show that introductions from multiple sources can contribute more to genetic variation than several introductions from the same source. Our results thus provide an empirical confirmation of the theoretical advice by Fuerst & Maruyama (1986) that large samples from many different sources are better than either small samples or samples from single sources. As our results exemplify, this even applies to a case like the Alpine ibex, where all the available source populations were derived from the same ancestral population. Thus, admixture represents a way to increase the genetic variation of reintroduced populations, which is often neglected because admixing source populations requires considerable more effort than using individuals from a single source. It is conceivable that admixture will also reduce the negative effects of repeated bottlenecks on population fitness. Several studies have documented substantial fitness loss after repeated bottlenecks (Thevenon & Couvet 2002; Leberg & Firmin 2008), although it is not a universal pattern (Bryant *et al.* 1999). The lower fitness of bottlenecked populations is generally attributed to inbreeding depression. Admixture between the different historical groups might lead to genetic rescue, because the groups are likely to carry different deleterious alleles. Several studies in wild populations have shown genetic rescue after the release of individuals from another population (Westemeier *et al.* 1998; Madsen *et al.* 1999; Hogg *et al.* 2006; Fredrickson *et al.* 2007). Furthermore, both higher founder population size and a higher number of source populations increased colonization success in an experimental study of waterstriders (Ahlroth *et al.* 2003) and

increased the establishment rate in introduced bird populations (Kolar & Lodge 2001).

Could admixture of animals from genetically distinct populations lead to outbreeding depression? Outbreeding depression has been documented in a wide variety of species (Templeton 1986; Edmands 1999; Marr *et al.* 2002), so its potential effects in admixed reintroductions cannot be dismissed (Marshall & Spalton 2000). However, in many reintroduced populations the risk of outbreeding depression is reduced because source populations often used to be part of the same metapopulation only a few generations ago, and genetic divergence might reflect drift rather than adaptation. Alpine ibex populations might fall into this category: they differentiated substantially in only 12 generations. In accordance with these considerations strategies for reintroductions advise to use founders from populations that historically exchanged genes (Moritz 1999).

In conclusion, our data show that releasing individuals from different source populations might be more important to maintain high genetic variation in a reintroduced population than releasing many individuals from a single source population. The strong effect of founder diversity on genetic variation is valuable information for future reintroductions of other species that experienced a similar history as Alpine ibex with a severe bottleneck and recovery in separate populations.

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Table 1: Reintroduction history and genetic parameters for each population. Information of reintroduction history is from Stuwe and Nievergelt (1991), from the ibex archive of the Federal Office for Environment and from hunting authorities.

population	pop. short	source	rel. Ind.	found. time	admix	N	He	Ho	Na
Adula-Vial	av	al, br	46	1963-1971	0.24	37	0.40	0.38	2.5
Piz Albris	al	ih, pp	42	1926-1934	0.50	61	0.41	0.42	2.5
Aletsch-Bietschhorn	ab	br, ih, pl, pp	57	1932-1979	1.25	43	0.47	0.46	2.9
Alpstein	ap	al, pp	12	1955-1956	0.69	30	0.35	0.34	2.3
Arolla	ar	pl	96	1960-2004	0.00	36	0.43	0.44	2.7
Bire-Oeschinen	bo	br	13	1961-1962	0.00	18	0.41	0.43	2.5
Brienzer-Rothorn	br	ih, pp	18	1921-1980	0.45	39	0.46	0.46	2.6
Calanda	ca	al	36	1968-1970	0.00	31	0.37	0.37	2.3
Churfirsten	ch	ap, gh, pl	27	1984-1993	0.87	24	0.41	0.42	2.6
Crap da Flem	cf	al	39	1958-1969	0.00	27	0.40	0.39	2.3
Dents du Midi	dm	pl	21	1961-1979	0.00	23	0.44	0.42	2.6
Ferret	fe	gh, pl	47	1962-1993	0.64	19	0.41	0.39	2.6
Fluebrig	fl	al, pl	21	1962-1971	0.60	32	0.39	0.39	2.5
Flueela	fu	al	42	1958-1987	0.00	21	0.36	0.35	2.3
Foostock	fo	gh	12	1927-1961	0.00	27	0.38	0.41	2.4
Gornergrat	go	ih, pl, se	10	1946-1990	0.90	23	0.40	0.38	2.6
Graue Hoerner	gh	al, ih, pp	55	1911-1961	0.63	47	0.40	0.41	2.5
Gross Lohner	gl	br, pl	33	1951-1968	0.67	22	0.46	0.44	2.9
Hochwang	hw	al	40	1965-1973	0.00	28	0.38	0.39	2.3
Julier Nord	jn	al	109	1954-1970	0.00	19	0.39	0.39	2.3
Julier Sued	js	al	41	1954-1970	0.00	23	0.39	0.37	2.4
Justistal	ju	br	25	1949-1957	0.00	19	0.41	0.43	2.4
Macun	ma	al	53	1969-1980	0.00	22	0.38	0.38	2.4
Mischabel	mi	pl	25	1960-1965	0.00	33	0.40	0.39	2.5
Muveran	mu	pl	58	1959-2001	0.00	27	0.38	0.39	2.6
Nufenen	nu	ab, pl	33	1975-1991	0.30	19	0.38	0.39	2.6
Oberbauenstock	ob	al	23	1969-1986	0.00	30	0.34	0.34	2.2
Pierreuse-Gummfluh	pg	al, br, dh, pl	14	1955-1965	1.30	41	0.47	0.49	2.8
Pilatus	pi	al	17	1961-1965	0.00	17	0.36	0.40	2.3
Pleureur	pl	gp, ih, pp	78	1928-2006	0.87	23	0.42	0.43	2.7
Rheinwald	rh	al	29	1954-1965	0.00	35	0.38	0.38	2.5
Rothorn-Weissfluh	rw	al	77	1959-1971	0.00	29	0.42	0.42	2.4
Schwarzmoench	sm	br, ih	26	1924-1950	0.69	32	0.45	0.44	2.7
Tanay	ty	pl	9	1977-1978	0.00	25	0.40	0.39	2.5
Umbrail	um	al	59	1970-1979	0.00	29	0.41	0.40	2.5
Val Bever	vb	al	137	1957-1971	0.00	32	0.40	0.38	2.5
Weisshorn	wh	pl	24	1962-1974	0.00	25	0.36	0.37	2.6
Weissmies	wm	ab, pl	31	1960-1982	0.38	49	0.39	0.39	2.7
Wetterhorn	we	br, ih, pp	21	1926-1964	1.00	19	0.42	0.43	2.4
Wittenberg	wb	br, pl	21	1958-1961	0.31	21	0.44	0.44	2.7
Mean			39		0.31	29	0.40	0.40	2.5

pop.short: abbreviation for each population; source: source population of released individuals (pp: wildlife zoo Peter&Paul, ih: wildlife zoo Interlaken Harder, dh: wildlife zoo Dählhölzli, se: wildlife zoo Seiler; NA: no information available); rel. Ind.: number of released individuals; found. time: time period of released individuals; admix: admixture index calculated with Shannon-Wiener index; N: number of samples; He: expected heterozygosity; Ho observed heterozygosity; Na: allelic richness

Table 2: Estimation of genetic founders for 22 Alpine ibex populations with NFCONE (Anderson & Slatkin 2007) and parameters used for the estimation.

population	pop.short	source	gen	carr.cap	gen.f	low CI gen.f	upp CI gen.f
Arolla	ar	pl	6	500	23	14	42
Bire	bo	br	6	100	20	11	43
Calanda	ca	al	5	100	12	7	19
Crap.Flem	cf	al	6	120	16	10	30
DentsMidi	dm	pl	6	500	21	12	43
Flueela	fu	al	6	400	21	12	46
Foostock	fo	gh	10	400	13	9	21
Hochwang	hw	al	5	180	15	9	26
Ju.Nord	jn	al	7	600	16	10	31
Ju.Sued	js	al	7	500	28	15	65
Justistal	ju	br	7	100	13	8	23
Macun	ma	al	5	200	18	11	33
Mischabel	mi	pl	6	650	30	16	76
Muveran	mu	pl	6	500	15	10	24
Obbauest	ob	al	5	250	7	5	9
Pilatus	pi	al	6	100	14	8	27
Rheinwald	rh	al	7	450	29	18	54
Roth.Weiss	rw	al	6	350	42	21	131
Tanay	ty	pl	4	400	11	7	19
Umbrail	um	al	5	150	15	10	24
Val.Bever	vb	al	6	300	78	32	>1000
Weisshorn	wh	pl	6	400	15	10	23

Pop.short: abbreviation of the popuations; source: source population; gen: number of generations; carr.cap: carrying capacity; gen.f: estimated number of genetic founders; low CI gen.f: lower 95% confidence interval of genetic founders; upp 95% CI gen.f: upper confidence interval of genetic founders

Table 3: Effects of parameters of the reintroduction history on expected heterozygosity (He) and number of Alleles (Na) for the three best models. Standardized coefficients (Coeff.) are shown.

Model	df	AICc	adj.R^2	Term	coeff.	P
He = fy + ax + gf	5	107.2	29.3	founder year	-0.121	0.439
				admixture index	0.512	0.002**
				combined founders	0.218	0.13
He = fy + ax + gf | random: source	6	108.7	27.3	founder year	-0.094	0.547
				admixture index	0.480	0.011*
				combined founders	0.248	0.061
He = fy + ax + rf	5	109.2	25.7	founder year	-0.161	0.307
				admixture index	0.471	0.005**
				released founders	0.102	0.474
Na = fy + ax + gf | random: source	6	87.7	32.5	founder year	0.107	0.453
				admixture index	0.566	0.003**
				combined founders	0.293	0.001**
Na = fy + ax + rf + fy*rf | random: source	7	91.1	25.3	founder year	0.083	0.564
				admixture index	0.621	0.002**
				released founders	0.211	0.030*
				founder year*released founders	0.203	0.081
Na = fy + ax + rf | random: source	6	91.2	29.3	founder year	0.065	0.654
				admixture index	0.520	0.006**
				released founders	0.259	0.009**

fy: founder year; ax: admixture index; rf: released founders; cf: combined founders; ***p<0.001; **p<0.01; *p<0.05

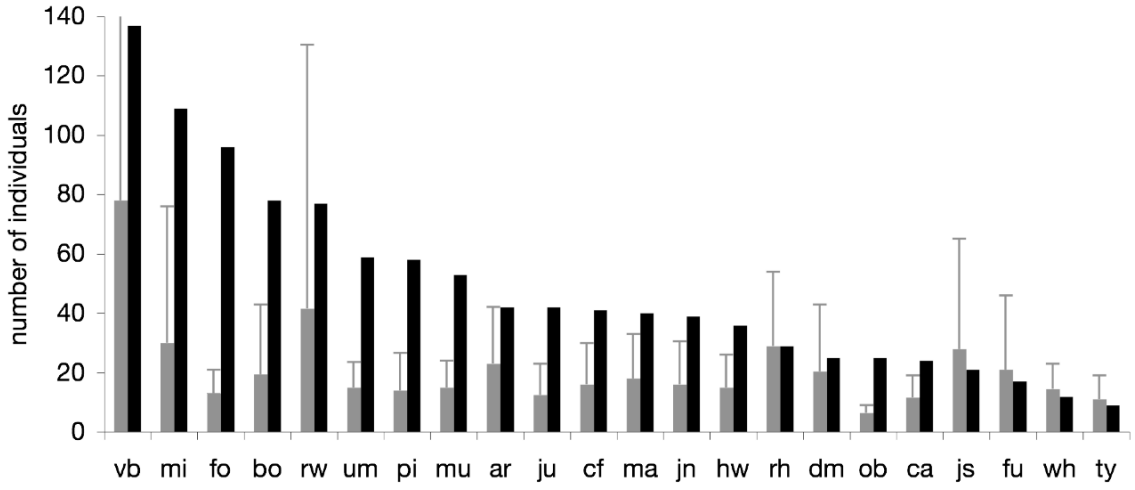

Figure 1: Number of released (black) and genetic founders (grey) for 22 Alpine ibex populations. Error bars represent 95% confidence limits of the genetic founders. The vb population had a 95% confidence limit above 1000.

3 INBREEDING AND EFFECTIVE POPULATION SIZE OF REINTRODUCED ALPINE IBEX

Iris Biebach & Lukas F. Keller

Abstract

Inbreeding and its detrimental consequences have gained attention in the recent years, because the number of small and isolated populations is increasing. Understanding the circumstances when most inbreeding is accumulating is central for conservation biology. However, exploring population parameters that may have influenced inbreeding requires that the population parameters and the estimate of inbreeding accumulation refer to the same time scale. Here we used 41 reintroduced Alpine ibex populations to decompose inbreeding into contemporary inbreeding and inbreeding that accumulated since the last common ancestral population. We used effective population size to estimate contemporary inbreeding and population specific Fst to estimate inbreeding that included the population history. We applied the latter method to two time periods: over the time since reintroduction of the populations to Switzerland (total inbreeding) and over the time since the last split (split inbreeding), when most populations were founded, on average 55 years after the initial reintroductions in Switzerland. Current census size (Nc) significantly influenced contemporary inbreeding, however, it explained only 16% of the variation among populations. Total inbreeding equaled on average that from one generation of half sib mating, however there was high variation among the populations. This variation was explained by the size and admixture of the founder group. Split inbreeding estimates were significantly influenced by the harmonic mean population size since founding of the population. Contemporary inbreeding influenced neither total inbreeding nor split inbreeding suggesting it does not reflect inbreeding levels in recently founded populations. This study emphasizes the importance of the composition of the founder group and the early growth rate after founding in reducing inbreeding in reintroduced populations.

Introduction

Inbreeding and its detrimental consequences have been recognized long ago, mainly by observation of inbred lineages in agriculture (Wright 1977). With the advent of conservation biology attention was also drawn to inbreeding in natural populations because small and isolated populations and populations that experienced bottlenecks or founder events are expected to have increased inbreeding levels. In the meantime, studies have shown that inbreeding may lead to reduced fitness and increased extinction risk also in wild populations (Newman & Pilson 1997; Keller 1998; Saccheri *et al.* 1998; Madsen *et al.* 1999).

In populations that are subject to conservation management, some population parameters that may impact inbreeding may be under (partial) control of the conservation biologists allowing them to take measures to minimize inbreeding. For example, in reintroduction programmes managers may have control over the timing and the number of founders released, two parameters that will affect the degree of inbreeding in the resulting population (Allendorf & Luikart 2007). To allow optimal management it is important to understand when most of the inbreeding is built up in a reintroduced population and which parameters are most influential. To do so, total inbreeding needs to be decomposed into estimates that refer to the same time scale as the parameters of interest.

In an idealized Wright-Fisher population inbreeding accumulates at a constant rate. However, natural populations are not ideal: they change in size, in sex ratio and in variance of reproductive success. Therefore, the accumulation of inbreeding is not constant over time (Chesser *et al.* 1993; Wang 2005). Particularly the small population sizes following a bottleneck or founder event are a key factor in contributing to inbreeding, because inbreeding increases proportional to the reciprocal of the population size (Gillespie 2004). In addition to founder population size the relatedness among the founders is expected to influence inbreeding in a reintroduced population. Finally, even a population that has recovered from a bottleneck will experience further inbreeding each generation due to its finite population size. This inbreeding may be non-trivial and adds each generation to the already existing inbreeding (e.g. Ewing *et al.* 2008). In this study, we aimed to decompose total inbreeding into the contributions of early

reintroduction history and contemporary inbreeding, and to identify those population parameters that may have influenced levels of inbreeding.

All inbreeding estimates are relative and thus there are several inbreeding estimates that differ in their reference populations (Jacquard 1975; Keller & Waller 2002). Different reference populations correspond to different time periods over which inbreeding has accumulated and they can therefore be used to decompose inbreeding levels of populations into the contributions from different time periods in the past (Jacquard 1974, page 169). If inbreeding occurs both due to subdivision in finite populations and non-random mating within subpopulations, the total inbreeding of a subpopulation is given by (1-Fit)=(1-Fis)(1-Fst) (Wright 1977). With random mating in the subpopulations, Fis is zero, and total inbreeding relative to that expected under random mating in the entire population equals Wright's Fst (Keller & Waller 2002).

The effective population size (Ne) can be used to estimate the contemporary rate of inbreeding per generation. The most widely used approaches to measure effective population size are variance and inbreeding Ne. While inbreeding Ne determines the rate of increase in homozygosity, the variance Ne measures the genetic drift resulting from one generation of genetic sampling (Wang 2005). In a population of constant size estimates of the two Ne approaches are identical, but in a population of changing size they differ (Chesser *et al.* 1993). We are primarily interested in the rate of inbreeding, however, existing methods to estimate Ne from genetic markers provide the variance Ne. Thus, in growing populations we will underestimate inbreeding if we calculate it using the variance effective population size. Early breeding experiments showed that inbreeding rates of 2-3% per generation often could be compensated by selection while higher rates were unsustainable (Stephenson *et al.* 1953). Assuming that inbreeding depression might be more pronounced in the wild than in controlled environments, a rule of thumb was developed from these findings that 1% of inbreeding might be sustainable in the long run in the wild, corresponding to an effective population size (Ne) of 50 (Franklin 1980; Soulé 1980).

The effective size of a population can be determined by life history data, where available. Without detailed life history information, Ne estimated from

demographic data might be inaccurate (Frankham 1995), because not all relevant factors will be taken into account. The main factors that determine Ne are population size and its fluctuations, variance in reproductive success and sex ratio (Nunney 1991, 1993; Frankham 1995). In contrast to demographic Ne estimates, genetic data can provide estimates of Ne that include all factors that influence Ne, but they have the disadvantage of large confidence intervals especially for larger effective population sizes (Nei *et al.* 1975; Luikart *et al.* 1999). Nevertheless, genetic data are often the only practical way to estimate effective population sizes of wild populations.

Here we estimate the effective population sizes and total and contemporary inbreeding in 41 populations of Alpine ibex (*Capra ibex ibex*). Alpine ibex is a successfully reintroduced species with many populations descending from one common ancestral population in northern Italy and the reintroduction history is well known for each population (Chapter 1). This allowed us to decompose total inbreeding into contributions from various phases of the reintroduction and to relate these inbreeding estimates to demographic parameters such as founder composition, current census population size and effective population size.

Methods

Populations and Samples

Between 2004 and 2007 we collected 1206 tissue and blood samples from both sexes and all age classes from 41 Alpine ibex populations across Switzerland with an average sample size of 29 (range: 18 to 61) per population (Table 1). For detailed information on population definitions and sampling see Chapter 1. For all populations current census sizes (Nc) of 2007 and for fewer populations yearly census size further back were as well provided by Swiss hunting authorities.

For 10 of the 42 populations we had in addition to the recent samples 258 tissue samples collected between 1986 and 1988 (Stuwe & Scribner 1989) (Table 1). Thus, for 10 populations we had samples from two sampling periods two to three generations apart, if we assume a generation time of 8 years in Alpine ibex (Grodinsky & Stuwe 1987).

Genetic data

All samples were genotyped at 37 neutral microsatellites as described in Chapter 1. Unreliable genotypes were repeated up to three times and only reliable genotypes were used for further analysis. We estimated allelic dropout and false allele rates for the first sampling period (1986-1988) by repeating between 9.3% and 46.2% of the samples per locus. We used a maximum-likelihood-based method implemented in PEDANT (Johnson & Haydon 2007) to estimate genotyping error rates, as we had done previously for the recent sampling period (2004-2007) (Biebach Chapter 1).

Contemporary effective population size

We estimated Ne using two methods: allele frequency changes through time (temporal method) and linkage disequilibrium at a single point in time (LD method). We used both methods because they make different assumptions and thus provide somewhat complementary answers. Both methods assume isolated populations without immigration, a reasonable assumption for most ibex populations as they live on mountain tops separated by geographic structures leading to no or low gene flow between populations (Maudet *et al.* 2004).

The temporal method uses the fact that in the absence of other forces such as migration, selection and mutation allele frequency changes over time are solely a function of genetic drift and can be used to estimate variance Ne (Wang 2001). The temporal method estimates the harmonic mean Ne for the time between the two temporal samples. There are several statistical approaches to estimate Ne from temporal samples that tend to give similar results in comparative studies (Aspi *et al.* 2006; Fraser *et al.* 2007). Thus we used only one temporal method, a Bayesian coalescent-based method implemented in CONE (Anderson 2005). We set the generation time between the two sampling periods to 2, calculated the likelihood for Ne ranging from 2 to 800 in steps of 2 and used 1000 Monte Carlo replications. We applied this method to the 10 populations with samples from the two sampling periods.

The LD method estimates Ne in a single sample of neutral loci from the LD that is generated from genetic drift in isolated populations with random mating

(Hill 1981). In populations of constant size the LD method gives the Ne of the parental generation (Waples 2005). In growing or declining populations LD is influenced by the last few generations, because it takes ca. 3-5 generations to reach a new asymptotic LD (Waples 2005). Therefore, in a population of changing size, the LD method reflects the harmonic Ne of the last few generations (Waples 2005). To estimate Ne from LD we used the software LDNE (Waples & Do 2008) that includes a small sample size correction. LD is biased downwardly if alleles at low frequencies are included in the samples (Hudson 1985). Simulations show that LD is not biased if allele frequencies below 0.05 are excluded (Hudson 1985; Waples & Do 2008) and hence we used only allele frequencies above 0.05 for the LD analysis. Ne was estimated for a random mating system and confidence intervals were estimated by the jackknife method (Waples & Do 2008). The LD method has the advantage of requiring only one sampling time and thus we estimated Ne by the LD method for all the 41 populations of the recent (2004-2007) sampling period.

Due to an extreme outlier (Figure 2) we used a Spearman rank correlation to compare the temporal and LD Ne estimators. We calculated the ratio of the two estimators to investigate any systematic difference between the two. For further analysis we used the Ne estimates of the LD method, because we had data from 41 rather than just 10 populations as with the temporal method. We used linear regression analysis to explain variation in Ne among populations as a function of census population sizes. We expected that the contemporary LD Ne estimates reflect the harmonic mean of the last few generations (Waples 2005)**,** because wild populations rarely have constant population sizes. The harmonic mean census population size of the last four generations (hm4gen) was available for only 26 populations. However, hm4gen correlated highly ($r = 0.97$, $p<0.001$) with the census size (Nc) of these 26 populations in 2007. Therefore, to be able to use data from all 41 populations, we used the census data of the year 2007 for comparisons with LD Ne. Additionally, often only current census sizes exist for populations and it is valuable information if there is a relationship between current Nc and Ne, even though Ne refers to a few generations back. While highly correlated, hm4gen was on average 15% below the census size 2007. This

difference has no effect in the analysis of variation in LD Ne, but it is critical for the ratio Ne/Nc. Therefore we calculated the ratio Ne/hm4gen for the 26 populations for which we had harmonic mean data to get an estimate of the degree to which Ne is reduced compared to Nc.

Inbreeding

To quantify inbreeding that accumulated since the establishment of the reintroduced ibex populations we estimated population specific Fst as described in Vitalis et al. (2001, equation 8). There is no inbreeding due to non-random mating in the ibex populations: Fis is not significantly different from 0 in any population (Chapter 1). Therefore, population specific Fst in the Alpine ibex populations quantifies the level of inbreeding that arose over time since the last common ancestral population. We estimated population specific Fst with the software 2mod (Ciofi *et al.* 1999). 2mod uses coalescent theory and Markov chain Monte Carlo (MCMC) simulations to calculate the relative likelihood of two demographic models, an equilibrium drift-migration model and a non-equilibrium drift model, given the allele frequencies of the populations. Initial analyses of our data revealed no support for the gene-flow model (support for gene-flow model was 0% from 450 000 iterations; data not shown) as expected given the history of the Alpine ibex populations. Therefore, using a slightly modified version of 2mod we fixed the analysis to the non-equilibrium drift model to estimate inbreeding relative to the last common ancestral population. The model assumes that the reciprocal of the mutation rate is much longer than the divergence time (Ciofi *et al.* 1999), which is a reasonable assumption for the Swiss ibex populations since they were founded no more than 12 generations ago.

To decompose total inbreeding into contributions from different phases of the reintroduction, we estimated population specific Fst over two time spans, once over the whole reintroduction period since the first releases from the zoo populations (total inbreeding) and once since the last split when many populations were founded several generations after the first populations were established (split inbreeding) (Figure 1). Fst estimates over the whole time span quantify inbreeding for all 41 Swiss populations relative to the zoo populations

approx. 12 generations ago. These estimates of total inbreeding represent inbreeding that accumulated over one (e.g. populations al, br, pl) and two founder events (those founded at the last split), respectively (Figure 1). We estimated inbreeding relative to the ancestral population before the last split (split inbreeding) with 20 populations that were founded from only one of three wild source populations (al, br, pl) four to six generations ago. Each of the three sources and its descendant populations was analysed separately in 2mod. We then pooled the results because they all quantify inbreeding that has accumulated over a similar time period. Split inbreeding estimates present inbreeding that accumulated since the second founder event for those populations that experienced two founder events. Split inbreeding was also calculated for the three populations that were the sources (al, br and pl) of the populations founded at the last split. For these three source populations, split inbreeding quantifies the inbreeding that accumulated over the same time but without an additional founder event (Table 1).

We further explored the effects of population specific parameters on total inbreeding. We have previously found that the number of founder individuals and the admixture index of the founder group influences genetic diversity (Chapter 2). The admixture index measures the diversity of source populations contributing to the founding individuals, where the number of founders from each source population is counted proportionally. For details see Chapter 2. We hypothesized that these parameters will also affect population specific inbreeding. Therefore, we used the natural logarithm of the founder population size, the admixture index and LD Ne as explanatory variables in multiple regressions. LD Ne estimates were included to investigate whether current effective population sizes reflect the amount of inbreeding in a population that accumulated over the entire reintroduction history.

The harmonic mean of the effective population sizes is expected to determine the amount of inbreeding over T generations as follows:

$$Fst = 1 - e^{-T/2Ne}$$ eqn.(1) (Crow & Kimura 1970)

We used eq. 1 to calculate Fst from the harmonic mean population sizes (hm Fst) for comparison with split inbreeding calculated from molecular data. We

restricted this analysis to split inbreeding because most of the populations were founded at the last split (Figure 1). We omitted the first five years after the initial releases in the calculations of hm Fst to avoid biases due to missing data (which were common in the first years after a release) and different release modes. For example, the harmonic mean would differ if all individuals were released in the first year or over two consecutive years, though the inbreeding and variance effective population size would be similar under the reasonable assumption of no reproduction in the first year. For the three source populations of the last split,we used only the time after the last split (mean founding year: 1961) to calculate hm Fst. We used multiple regressions to relate split inbreeding to hm Fst and the LD Ne. For all multiple regression analyses, we used standard model reduction and omitted terms with $p>0.2$.

With the estimates of total inbreeding and split inbreeding we were able to decompose total inbreeding into the inbreeding that accumulated up to the last split (inbreeding before last split) and since the last split (split inbreeding). These two time periods correspond to approximately the first and last six generations of the time since the initial reintroductions. Inbreeding that accumulated before the last split was calculated from total and split inbreeding using the relationship:

$(1\text{-Fst.total}) = (1\text{-Fst.}1^{st})\,(1\text{-Fst.}2^{nd})$ eqn. (2) (Jacquard 1974, p. 169)

where Fst.total is total inbreeding, $Fst.1^{st}$ is inbreeding up to the last split and $Fst.2^{nd}$ is split inbreeding.

Unless stated otherwise, all statistical analyses were performed in the software package R, version 2.8.0 (R Development Core Team 2006).

Results

Genetic data of first sampling period (1986-1988)

Dropout rate per genotype for the 1986-1988 sampling period was between zero and 23.8% (mean: 3.4%) and the false allele rate per genotype was up to 5.3% (mean: 0.5%) for the 37 microsatellite loci (Appendix 1). Six markers had dropout rates above 5% and three of these had false allele rates above 1%. Only one marker, BM1225, had a false allele rate above 1% but no dropout errors. This locus and loci with dropout error rates above 5% were omitted from both

sampling periods for the analysis of effective population size with the temporal method. Genotyping error rates were much lower in the 2004-2007 samples (generally below 1%, see Chapter 1) and thus all loci were retained in the analyses of those samples.

Contemporary inbreeding

Effective population size could be estimated for eight of the ten populations with the temporal method (Table 1). For two populations (mi and pl) the maximum likelihood value was not within the investigated range of 2 to 800 and thus we did not get an Ne estimate for these two populations. However, the true value of Ne should be in this range, because census size of the two populations is about 600. There was so little genetic differentiation between the two sampling periods for these two populations (pairwise Fst between the two sampling periods: -0.006 and 0.0113, respectively) that the lack of an estimate could be explained by the fact that there is random sampling (manual of CONE, Anderson 2005). For the remaining eight populations the maximum likelihood Ne from the temporal method was between 22 and 651 with a mean of 135. Three of these estimates had infinite upper confidence intervals.

Two populations (vb and wh) also failed to give estimates of the effective population size from the linkage disequilibrium method, but these were not the same populations that failed to give estimates with the temporal method (Table 1). The two populations yielded negative Ne estimates because LD due to sampling error was higher than LD from drift in these two populations (Bartley *et al.* 1992). A larger sample size might result in reliable estimates, because increasing sample size will reduce the LD arising from finite sampling relative to the true signal of drift in LD. In the other populations, effective populations size estimates from the LD method ranged from 10 to 877 with a mean of 102 and eleven estimates had infinite upper 95% confidence intervals. 17 of the 39 Ne estimates were below 50 and 12 of these had also upper confidence intervals below 50. LD Ne estimates of three populations (av, gl and pl) were above the census size, but lower confidence intervals included the census population size.

There was no significantly positive correlation between the two methods of estimating contemporary effective population size (Figure 2, r = -0.64, p = 0.1) However, confidence intervals of the two methods overlapped for all populations but for the jl and bo population. While the jl population had a lower, the bo population had a higher temporal Ne than LD Ne estimate. The mean ratio of the LD method to the temporal method was 1.18 (95% CI: -0.52, 2.88). Thus there seemed to be no systematic difference in the estimated effective population size of the two methods. For further analysis involving contemporary effective population size we used the estimates of the LD method because we had data from 39 populations instead of the 8 with the temporal method.

Contemporary effective population size was significantly influenced by the census size in 2007 (Figure 3, b=0.33, F= 8.3, P=0.007). Two populations, al and pl were most influential in this regression, but removing them from the analysis did not substantially alter the results. However, Nc explained only 16% (24% with the two outliers excluded) of the variation in Ne. The mean ratio between the Ne estimates and the harmonic mean population size over the last four generations (hm4gen) was 0.58. When unrealistic values where Ne was higher than Nc were removed, the mean ratio was 0.34 (range: 0.1 to 0.75).

Contributions to inbreeding of various phases of the reintroduction

Mean total inbreeding since the zoo populations was 0.125 (sd ± 0.04), but inbreeding varied greatly (up to 83%) among the populations (Table 1). The total Fst values (total inbreeding) estimated with the likelihood approach in 2mod were only moderately (r=0.72) correlated with the Weir & Cockerham type estimator (Weir & Cockerham 1984) of Fst (data not shown). Such differences between moment based and maximum likelihood Fst estimators are commonly observed, but poorly understood (Beaumont, pers. comm.). LD Ne did not influence total inbreeding and was therefore omitted from further multiple regression models. Founder group size and admixture of the founder group had a significant impact on total inbreeding (Table 2). The impact of admixture on total inbreeding was 30% higher than the one of founder group size.

Split inbreeding was 0.067 (sd ± 0.028) and varied similarly among the populations (84%) as did total inbreeding. Hm Fst had a significant impact on split inbreeding (Table 2, Figure 4) and explained 44% of the variation among the populations. As was the case for total inbreeding, LD Ne did not explain variation in split inbreeding among populations.

Mean inbreeding for the time before the last split was very similar to the time after the last split (0.068±SD 0.019 vs 0.067± SD0.028) (Figure 5). Consequently, the ratio of inbreeding before the split to inbreeding after the split (Fst.1st/Fst.2nd) had a mean of 1.389, with a 95% confidence interval between -0.86 and 3.64. Thus, while the contribution to total inbreeding before and after the last split was the same on average, it varied greatly among populations and was higher for some populations before the split and for others after the split.

Discussion

Contemporary effective population size

To estimate contemporary Ne with the temporal method we used samples collected more than 20 years ago. These samples had on average 3.4-fold higher dropout and 5.5-fold higher false allele rates than samples of the 2004-2007 sampling period (Chapter 1). The higher error rates are likely the consequence of repeated thawing and freezing and radioactive radiation due to customs regulations when samples were transferred between countries (Scribner, pers. comm.). Accordingly estimates of Ne with the temporal method were based on fewer loci, because loci with high error rates were excluded.

Estimates of contemporary Ne using the temporal and the LD method were not correlated across the eight populations for which we had estimates from both methods (Figure 2), but Ne values were of similar magnitude. This is in line with a comparative study of Ne estimators (Fraser *et al.* 2007) where temporal and LD methods gave estimates of the same magnitude but were uncorrelated within populations. The ratio of the LD Ne to the temporal Ne of the ibex populations was also similar to the ratio reported by Fraser et al. (Fraser *et al.* 2007) for isolated populations. The mean recent effective population size of 102 translates into an increase in homozygosity of 0.5% per generation; for 17 populations this

value was more than 1%. Thus, 17 of 39 ibex populations are below the effective population size of 50 that is thought to be sustainable in the wild in the long run (Stephenson *et al.* 1953). There might be a downward bias in our Ne estimates, if there is gene flow or admixture, which leads to an increase in linkage disequilibrium relative to the one caused by genetic drift alone (Nei & Li 1973; Fraser *et al.* 2007). However, possible source populations of migrants in the ibex populations in our study have usually the same ancestral population as the recipient population. Thus, possible migrants are genetically similar (Chapter 1) and any downward bias of Ne would be small (Fraser *et al.* 2007). In addition to ongoing gene flow, admixture of the founder group might influence LD. However, LD decays at a rate of 0.5 per generation for unlinked loci**.** Most ibex populations in this study were founded ca. 6 generations ago. Thus, only 1.6% of the LD among unlinked loci should be due to admixture of the founder group.

The mean ratio of Ne to hm4gen among ibex was 0.58 and thus higher than comparable values in other mammals: Frankham (1995) reported a mean of 0.35 in mammlian studies where only variance in family size and sex ratio affected the Ne/Nc ratio. These should be the two main factors responsible for variation in the Ne/Nc ratio in Alpine ibex, because fluctuating population size is taken into account by dividing with hm4gen. However, if we exclude populations with a ratio above one the ratio is similar as reported for other mammals (Frankham 1995)**.** In Alpine ibex only few males have access to females, leading to a high variance in reproductive success of males and in turn to a reduced Ne as it is common for polygynous species (Hoelzel 1999; Stiver *et al.* 2008). There is the possibility that the Ne to Nc ratio is biased upwards, if LD is influenced by less than the last 4 generations, because the harmonic mean population size over less than 4 generations was higher than hm4gen. Additionally the real populations sizes are probably higher than the census counts, because censuses in this species are more likely to miss some animals than to double count individuals. Thus, undercounting might have caused an upward biased in the Ne to hm4gen ratios.

Current census size and Ne were positively correlated (Figure 3), suggesting that one could predict the effective population size from the census size. However, only 16% of the variation in Ne was explained by the current census

size suggesting that other factors also contribute to variation in Ne. The remaining variation was also reflected in the high range of Ne/Nc values among the populations. Factors such as variance in family size or sex ratio might vary among populations due to habitat and density differences and hence the ratio of Ne to Nc might differ. For example, some studies have reported an increased variance in reproductive success in larger populations (Ardren & Kapuscinski 2003; Hedrick 2005; Stiver *et al.* 2008). Additionally, harvest strategy and intensity differs between the ibex populations and might affect Ne differently (Ryman *et al.* 1981; Allendorf *et al.* 2008). However, Ne/Nc estimations are often variable among populations within a species (Waples 2002; Ardren & Kapuscinski 2003), suggesting that Ne/Nc ratios cannot be assumed constant over time or space (Palstra & Ruzzante 2008).

Contributions to inbreeding of various phases of the reintroduction

Substantial inbreeding accumulated in ibex populations during the entire reintroduction history since the breeding programmes in the zoos. The mean inbreeding coefficient was equivalent to one generation of half sib mating. Note that this does not imply that half sib matings are taking place. Instead, this value reflects the accumulation of inbreeding over time in small populations without mating between close relatives (e.g. Ewing *et al.* 2008).

Differences in inbreeding levels among the ibex populations could be explained by differences in founder group size and admixture of the founder group (Table 2). Thus, less inbreeding resulted when populations were founded with more individuals and with individuals from different populations. At the same time these parameters increased genetic diversity in these populations, measured either as number of alleles or expected heterozygosity (Chapter 2). However, the relative impact of the number of released individuals was higher on inbreeding than on genetic diversity. This emphasizes that while inbreeding and heterozygosity are closely related concepts they are not identical (Thompson 1976). The harmonic mean population size is one important factor determining inbreeding (see eqn. 1) and accounted for 44% of the variation in inbreeding in the Alpine ibex populations (Figure 4). This finding reiterates the importance of

fast growth after founding of populations in order to maintain genetic diversity (Nei *et al.* 1975) and reduce inbreeding. However, more than half of the variation in inbreeding since the last split was attributable to other, unknown population specific factors.

Contemporary effective population size did not reflect inbreeding of the ibex populations for both time periods, for total inbreeding and last split inbreeding (see Figure 1). Thus, estimates of the current effective population size do not reflect overall levels of inbreeding in reintroduced populations. We decomposed total inbreeding since the ancestral zoo populations into two time periods each ca. 6 generations long, representing the time before and after the last split. Ibex populations exhibited high genetic drift in the time before the last split (Chapter 1) and therefore we expected more inbreeding to accumulate in the first time period. However, the mean ratio of genetic drift before and after the split did not differ from 1.

Conclusion

Estimates of effective population size from linkage disequilibrium produced sensible results with ratios of effective to harmonic mean population sizes (Ne/hm4gen) that are comparable to other ungulates (Frankham 1995). Differences in the ratio among populations reflect different rates of inbreeding per generation. There is additional inbreeding from the demographic history of the reintroduction due to founder effects and subsequent population growth. These total inbreeding estimates were not correlated with contemporary Ne. Thus, contemporary Ne could not be used to predict levels of inbreeding in recently introduced populations. Instead, total inbreeding was more strongly affected by admixture of the founder group, founder group size and genetic drift.

It is desirable to reduce inbreeding in reintroduction programmes, which might be achieved in three different phases of the reintroductions: First, during the founding process inbreeding can be reduced if a large number of founder individuals and founder individuals from different source populations are used. Second, fast population growth following the founder event increases the harmonic mean population size that in turn reduces inbreeding. Third, increasing

population size and thereby the effective population size when the populations are well established, reduces inbreeding that is generated in addition to the inbreeding from the founder history. This last phase will gain more influence through time relative to the inbreeding from the founding history.

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Table 1: Population parameters for 41 Alpine ibex populations

Population	pop. short	ss 2004-2007	ss 1986-1988	founder events	Tem. Ne	LD Ne	tot. inbr.	split inbr.
Ad.Vial	av	37	NA	mixed	NA	298	0.087	NA
Albris	al	61	23	1	50	84	0.111	0.018
Alet.Biet	ab	43	NA	mixed	NA	75	0.068	NA
Alpstein	ap	30	22	mixed	651	54	0.191	NA
Arolla	ar	36	NA	2	NA	27	0.091	0.049
Bire.Oesch	bo	18	27	2	86	24	0.120	0.057
Br.Rothorn	br	39	26	1.000	61	81	0.120	0.050
Calanda	ca	31	NA	2	NA	26	0.187	0.111
CapeMoine	cm	49	NA	mixed	NA	120	0.125	NA
Churfirst	ch	24	NA	mixed	NA	19	0.106	NA
Crap.Flem	cf	27	NA	2	NA	27	0.154	0.098
Dents.Midi	dm	23	NA	2	NA	85	0.120	0.062
Ferret	fe	19	NA	mixed	NA	10	0.101	NA
Fluebrig	fl	32	NA	mixed	NA	51	0.172	NA
Flueela	fu	21	NA	2	NA	181	0.147	0.061
Foostock	fo	27	NA	mixed	NA	65	0.136	NA
Gornergrat	go	23	NA	mixed	NA	19	0.118	NA
Gr.Hoerner	gh	47	31	mixed	108	50	0.113	NA
Gr.Lohner	gl	22	NA	mixed	NA	254	0.037	NA
Hochwang	hw	28	NA	2	NA	47	0.153	0.083
Ju.Nord	jn	19	NA	2	NA	173	0.154	0.072
Ju.Sued	js	23	25	2	22	55	0.112	0.041
Justistal	ju	19	NA	2	NA	98	0.141	0.106
Macun	ma	22	NA	2	NA	28	0.124	0.058
Mischabel	mi	33	15	2	NA	355	0.137	0.073
Muveran	mu	27	NA	2	NA	37	0.114	0.081
Nufenen	nu	19	NA	mixed	NA	70	0.111	NA
Oberbauestock	ob	30	NA	mixed	NA	20	0.223	NA
Pierr.Gumm	pg	41	NA	mixed	NA	72	0.078	NA
Pilatus	pi	17	NA	2	NA	18	0.165	0.094
Pleureur	pl	23	47	1	NA	877	0.087	0.024
Rheinwald	rh	35	NA	2	NA	48	0.146	0.058
Roth.Weiss	rw	29	NA	2	NA	101	0.114	0.035
Schwmoench	sm	32	17	mixed	58	61	0.071	NA
Tanay	ty	25	NA	2	NA	27	0.162	0.109
Umbrail	um	29	NA	2	NA	22	0.114	0.088
Val.Bever	vb	32	NA	2	NA	NA	0.106	0.024
Weisshorn	wh	25	NA	2	NA	NA	0.141	0.099
Weissmies	wm	49	NA	mixed	NA	215	0.107	NA
Wetterhorn	we	19	NA	mixed	NA	15	0.181	NA
Wittenberg	wb	21	25	mixed	43	87	0.062	NA

pop.short: short name for the population; ss 2004-2007: sample size of the recent sampling period; ss 1986-1988: sample size of the first sampling period; temp.Ne: Ne estimated with the temporal method; LD Ne: Ne estimated with the LD method; tot. inbr.: total inbreeding; split inbr.: split inbreeding

Table 2: Effects of population history on total inbreeding and split inbreeding. Regression coefficients were standardized. ***p<0.001; **p<0.01; *p<0.05

inbreeding parameter	adj. R^2	term	F	p	coeff
total inbreeding	0.21	ln founder size	4.92	0.033 *	-0.328
		admixture	8.34	0.007 **	-0.427
split inbreeding	0.44	hm Fst	10.45	0.008**	0.698

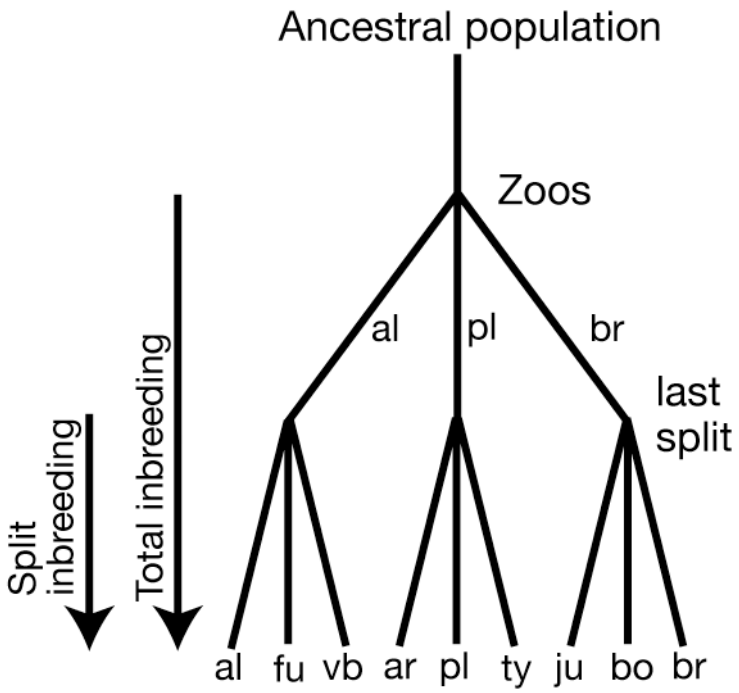

Figure 1: Schematic diagram of the reintroduction history of Alpine ibex showing only a subset of all populations. Population specific Fst was estimated over two time periods: since the zoo populations (total inbreeding) and since the last split of the populations (split inbreeding).

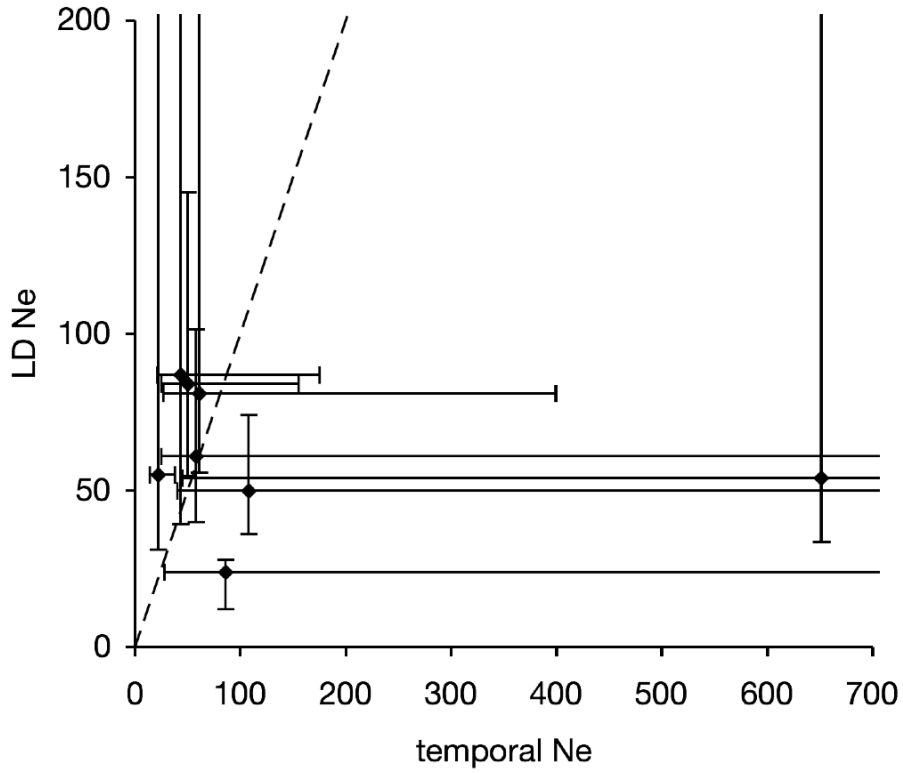

Figure 2: No correlation between contemporary effective population sizes estimated with the temporal method (temporal Ne) and linkage disequilibrium method (LD Ne). Dotted line represents the 1:1 ratio.

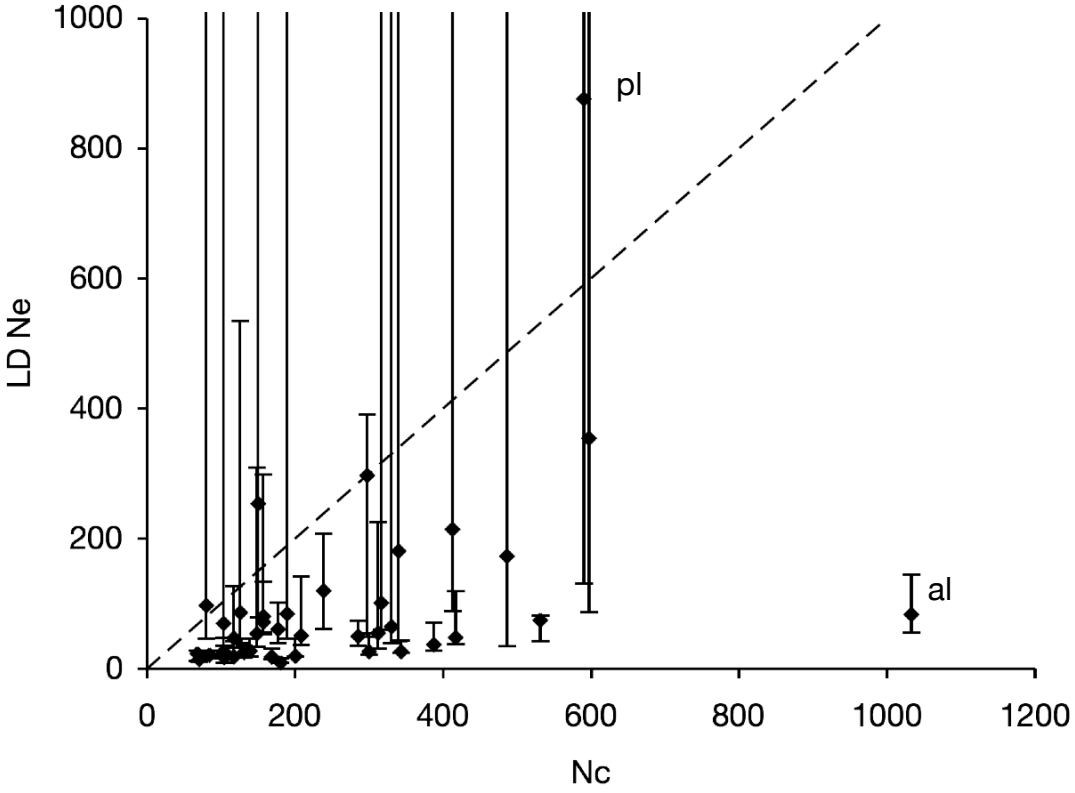

Figure 3: Census size of 2007 (Nc) significantly influenced contemporary effective population size measured by the LD method (LD Ne). (b=0.33; F=8.3 ; R^2=0.16 ; P=0.007)

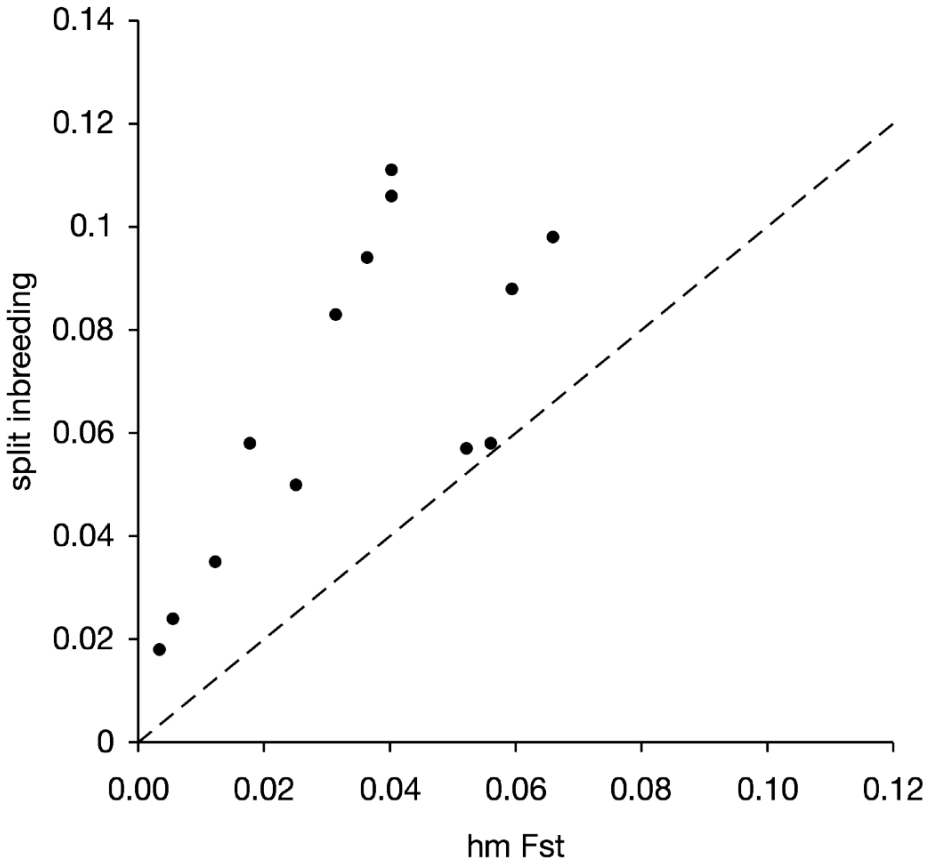

Figure 4: Genetic estimates of inbreeding since the last split (split inbreeding) depended on the harmonic mean population size since founding, transformed to Fst (hm Fst). The dotted line corresponds to the 1:1 ratio.

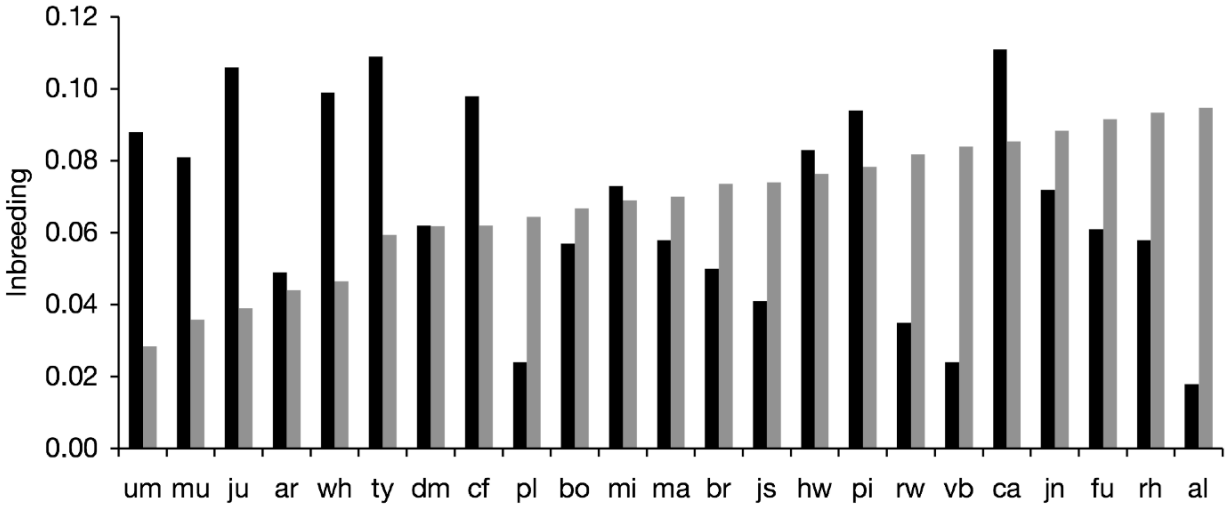

Figure 5: Inbreeding measured as population specific Fst before the last split (grey) and after the last split (black) for 24 reintroduced Alpine ibex populations.

Appendix 1: Estimated genotyping error rates for samples of the first sampling period (1986-1988).

Locus	Dropout	False
BM1225*	0.000	0.046
BM2113	0.048	0.000
BM302	0.000	0.000
BM415	0.024	0.000
BM4505*	0.090	0.000
CSSM47	0.000	0.004
HAUT27*	0.127	0.044
IDVGA30	0.000	0.008
ILSTS29	0.031	0.000
ILSTS30	0.034	0.000
INRABERN172	0.020	0.000
INRABERN175*	0.106	0.000
INRABERN185*	0.081	0.053
JMP29	0.017	0.000
MAF209	0.000	0.000
MAF36	0.000	0.000
MAF70	0.000	0.000
McM152	0.000	0.000
McM173	0.000	0.000
MILSTS076	0.046	0.000
OarAE54	0.030	0.000
OarFCB193	0.000	0.000
OarFCB20	0.000	0.000
OarFCB48	0.000	0.000
OARHH35	0.012	0.000
OarVH34	0.050	0.000
SR-CRSP01	0.019	0.000
SR-CRSP08*	0.238	0.046
SR-CRSP09	0.034	0.000
SR-CRSP23	0.036	0.000
SR-CRSP24	0.049	0.000
SR-CRSP25	0.024	0.000
TGLA10	0.009	0.000
TGLA122	0.047	0.000
TGLA126	0.000	0.000
TGLA73	0.000	0.000
URB058*	0.071	0.000
mean	0.034	0.005

4 INBREEDING EFFECTS ON POPULATION DYNAMICS

Iris Biebach, Marc Kéry, Michael Schaub, Lukas F. Keller

Abstract

Small populations are inherently at risk of stochastic fluctuations and inbreeding. However, while there is evidence that inbreeding reduces individual fitness the impact on population dynamics such as population growth rate is less clear. Inbreeding depression in individual fitness may not result in a reduced population growth rate if selection is density- and frequency-dependent. Here we estimated population growth rates and density dependence in 16 reintroduced Alpine ibex populations with a state-space model in a Bayesian framework. We did not find a clear effect of inbreeding on population growth rates. However, there was a trend towards reduced growth rates among populations with higher inbreeding. We found little evidence for density dependence indicating the potential that inbreeding depression could lead to reduced growth rates in Alpine ibex populations. However, it remains to be studied whether inbreeding truly decreases population growth rate and if it is a common pattern.

Introduction

Small populations are inherently at risk of stochastic fluctuations and inbreeding. The synergistic interactions of environmental stochasticity, demographic stochasticity and genetic effects have been proposed to cause the extinction of small populations (Gilpin & Soulé 1986). However, the importance of genetic effects in extinctions is still controversial (Lande 1988; Spielman *et al.* 2004). This is, in part, due to the fact that inbreeding and its detrimental consequences have mostly been studied at the individual level: Inbreeding depression in individual fitness is well established in laboratory, captive, and natural populations (e.g. Ralls & Ballou 1986; van Oosterhout *et al.* 2000; Keller & Waller 2002; Kristensen & Sørensen 2005) and has been demonstrated in many fitness and fitness related traits such as immune response, reproductive success and survival (e.g. Keller 1998; Slate *et al.* 2000; Reid *et al.* 2003, for examples of natural populations). However, inbreeding depression in individual fitness components is of limited importance to conservation biology unless the reductions in individual fitness translate into reduced populations growth rates (Keller *et al.* 2007). There is indirect evidence that inbreeding can reduce population growth rates from studies of extinction rates of inbred Drosophila lines (Bijlsma *et al.* 2000), experimental plant populations (Newman & Pilson 1997) and a natural butterfly metapopulation (Saccheri *et al.* 1998). In addition, other studies show a reversal of negative population growth rates by experimentally restoring immigration in inbred populations (Westemeier *et al.* 1998; Madsen *et al.* 1999; Vila *et al.* 2003; Hogg *et al.* 2006).

Small and inbred populations do not always, however, experience reduced population growth rates (Hoelzel *et al.* 1993; Broders *et al.* 1999). The strongest argument why inbreeding depression in individual fitness does not always translate into a reduced population growth rate is soft selection (Saccheri & Hanski 2006). Soft selection implies that the selection is density- and frequency-dependent (Wallace 1975), that is survival and reproductive success of an individual may depend on the presence or absence of other individuals. For example, in species with limited resources such as breeding sites, territories or access to mating partners, the strongest competitors within a population will

generally use these resources. In the context of inbreeding depression this implies that the least inbred individuals within a population use the resources. In large populations this might be outbred individuals but in small populations these individuals might be appreciably inbred. In the absence of any fitter competitors, these inbred individuals may produce enough offspring so that inbreeding depression in individual fitness has negligible effects on population size (Wallace 1970, 1975).

In contrast, hard selection is density- and frequency-independent. One example of hard selection are lethal genes that kill their carriers under all known conditions (Wallace 1975). If unconditionally lethal genes are a major source of inbreeding depression, then hard selection predominates and inbreeding depression in individual fitness would reduce population size. However, purging is likely to remove lethal genes from many populations and a substantial part of inbreeding depression in individual fitness traits is expected to be caused by genes of minor effect (Willis 1999). Thus, the evidence we have on the genetic architecture of inbreeding depression to date suggests that hard selection is not an inevitable consequence of inbreeding. Furthermore, the outcome of inbreeding depression may be dependent on environmental stressors. Synergistic effects of inbreeding and environmental stressors are not only known from individual fitness traits (Coltman *et al.* 1999; Keller *et al.* 2002; Armbruster & Reed 2005), but also from extinction probabilities in the laboratory (Bijlsma *et al.* 2000). Environmental stressors may thus change the genetic architecture of inbreeding depression to one favouring hard selection (Keller *et al.* 2007).

Empirical studies on natural populations that investigate inbreeding depression in population growth rates are scarce (Reed *et al.* 2007). Here we investigate this issue in Alpine ibex populations that vary in their degree of inbreeding (Chapter 3). One difficulty of such analyses is that population growth rates are influenced by factors other than inbreeding, first and foremost among them environmental conditions (Morris & Doak 2002). When such influences are not taken into account, spurious associations between inbreeding and population growth rates might result. For example, spurious correlations might appear if analyses cover different geographical regions that differ in habitat quality or

environmental conditions. Populations living in habitats with low quality might have always had low growth rates and therefore smaller population sizes than populations in high quality habitats. Most inbreeding is accumulated during times when population sizes are low, because homozygosity increases each generation proportional to the reciprocal of the effective population size (Gillespie 2004). Thus, low growth rates might cause inbreeding levels to be high and lead to the conclusion that inbreeding affects the population growth rate, while truly growth rate is low because of other factors such as reduced habitat quality instead of inbreeding. This problem can be addressed by testing for a relationship between the population growth rate in the first years after a founder event and the growth rate in later years. If there is a correlation between the two time periods, i.e. populations had low growth rates in the initial and later years, nothing can be said about the likely direction of causality. However, if there is no correlation between the two time periods it is likely that inbreeding affected population growth rates and not vice versa.

Here, we used time series data of 19 Alpine ibex populations since their reintroduction to estimate population growth rates and to explore possible impacts of inbreeding on growth rates. To investigate the direction of causality we estimated the correlation between population growth rates in the first and last 30 years. Since inbreeding accumulates over the generations after a population has been reduced to small size (Keller *et al.* 2001; Ewing *et al.* 2008), the effects of inbreeding may only be seen after several generations. Thus, we used the population growth rates of the last 30 years to quantify inbreeding depression.

Methods

Study populations and census data

Alpine ibex were extirpated from the Alps by the end of the 19th century and only one Alpine ibex population survived in the Gran Paradiso region in northern Italy. During the last 100 years Alpine ibex were successfully reintroduced to many parts of Switzerland. However, animals were generally not directly translocated from the last wild population, but were first bred in zoos and then released into wild habitat. Such reintroduction procedures lead to founder events that increase

inbreeding (Chapter 3) and reduce genetic diversity (Stuwe & Scribner 1989; Chapter 1) For a detailed description of the history of the Swiss ibex populations see Chapter 1. After the reintroduction, populations were monitored closely (Bachler 1935; Nievergelt 1966) with yearly census counts conducted usually in springtime. During springtime ibex are found in the restricted areas with fresh vegetation without snow but generally still above the timberline (Abderhalden 2004) and are therefore easier to count than most other ungulates. Census data represent the number of ibex alive before reproduction in June and after the winter when most mortality takes place. Harvest of the first ibex populations started in 1977 when many populations had grown to high densities. Hunting takes place in autumn before the rut, which is in December and January (Aeschbacher 1978). Thus hunted females and hunted males do not contribute to offspring in the following year.

We had yearly census data from founding until the year 2007 for 19 Alpine ibex populations (Table 1). In some years census data were missing in some populations, particularly in the years following the reintroduction event. Though the census size was not documented in these instances, game wardens often know how the population developed. We used game wardens' knowledge to recontruct the missing data of two populations (pi and ob) for the first years after the release. Time series length varied between 24 and 97 years with a mean of 53 years. 10 populations had between 2.3% and 50% missing data (Table 1). Additionally, we had data on the number of harvested animals per year and, for some years, on the number of individuals found dead.

Population model

We used a logistic population model to estimate population growth (r), strength of density dependence (b) and environmental stochasticity (env). Given the true population sizes N at time t and t+1, the model is

$$\ln (N_{t+1}) = \ln (N_t) + r + b * N_t + env_t \qquad \text{(eqn 1)}$$

where env is a normally distributed random variable with mean 0 and variance σ^2_{env}. (Grotan *et al.* 2008). This model estimates the specific deterministic growth rate, which is the growth rate at very low population sizes (Saether et al. 2007). However, released and hunted animals had to be taken into account to get unbiased estimates of the population dynamic parameters. We followed the approach of Saether et al. (2007) to account for the harvested animals. Contrary to Saether (2007) and Grotan (2008) we also included years with releases of animals, accounting for these releases in the same way that we accounted for harvested animals. Thus, we adjusted the model in the following way:

$$\ln (N_{t+1}) = \ln (N_t - H_t + R_t) + r + b * (N_t - H_t + R_t) + env_t \qquad \text{(eqn 2)}$$

where R_t and H_t are the number of released and hunted individuals, respectively, in year t. We subtracted hunted individuals in year t from the population size in year t because they do not exist anymore for the census count in year t+1 and do not contribute to offspring in the year t+1. Released individuals were not included in the census count of the year t but potentially contribute to offspring of the year t+1. Thus we added the released individuals to the census of year t. The winter 1998/99 was an exceptionally harsh winter for ibex, because of heavy snow falls at the end of the winter and many ibex died in avalanches or starved to death (game wardens, pers.comm). This study aimed to get accurate estimates of the specific deterministic growth rates that might be biased if such crashes in the population size are not taken into account. Similarly, in the years 1991 and 1993 severe disease outbreaks of Keratoconjunctivitis occurred in the rw population. Thus, animals that were found dead in springtime following a winter with these severe environmental conditions (winter crash or disease outbreak) can be considered extraordinary mortalities and can be treated in the same way as harvested animals. Therefore, we added these mortalities to the shot animals of the previous year.

Because our data are count data and true population sizes (N) are unknown we used a state-space model (De Valpine & Hastings 2002) fitted to the population counts (Y). A state-space model can be regarded as a hierarchical

model (Royle & Dorazio 2008) with two dependent processes. The first process is the observation process, which links the observations (Y) to the true, but unknown state (population size) under consideration of an observation error (obs).

$$Ln(Y_t) = \ln(N_t) + obs_t \qquad \text{(eqn 3)}$$

where obs is described by a normally distributed random variable with mean 0 and variance σ^2_{obs}. Modelling the observation process with a normal distribution on the log-scale is realistic for many ecological sampling protocols (Dennis *et al.* 2006), because it explicitly considers that the magnitude of error increases with increasing population size.

Estimation of population parameters

We fitted models in WinBUGS (Lunn *et al.* 2000) called from R with R2WinBUGS (Sturtz *et al.* 2005). WinBUGS calculates the posterior distributions of the parameters of interest using Markov Chain Monte Carlo simulations (MCMC). We checked convergence of the MCMC simulations using the Gelman-Rubin-Brooks diagnostics (Brooks & Gelman 1998). Convergence was usually obtained after 700 000 iterations. We therefore ran 900 000 iterations, discarded the first 700 000 samples and thinned the remaining ones to 10.

We used normally distributed priors for growth rate and density dependence and uniform priors for environmental stochasticity and observation error. Priors were:

$$r, b \sim N(0, 0.001)$$
$$\sigma^2_{obs}, \sigma^2_{env} \sim \text{uniform}(0,100)$$

To investigate the direction of causality, we estimated the growth rate independently for the first 30 years after reintroduction and for the last 30 years of the time series. We did not simply split the time series length in half because many populations did not converge if shorter time periods were used. Therefore, time series of the first and last 30 years overlapped partially in 13 populations and

completely in one population (ch). The latter was therefore removed in the Pearson correlation comparing the two time periods. For further analysis the estimates of r, b, obs and env of the last 30 years of a time series were used. The proportional component of variance due to process noise, Φ_1 was expressed as (Dennis *et al.* 2006)

$$\Phi_1 = \sigma^2_{env} / (\sigma^2_{env} + \sigma^2_{obs}) \qquad \text{(eqn 5)}$$

Inbreeding

An average of 29 (range 17–61) individuals in all 19 populations were genotyped at 37 neutral microsatellite loci. For detailed information about the microsatellite loci and genotyping protocols see Chapter 1.

Inbreeding was estimated using a population specific Fst calculated in 2MOD (Ciofi *et al.* 1999) as described in Chapter 3. Population specific Fst measures inbreeding due to population subdivision and is relative to the population ancestral to all the populations in the analysis. In our case, this ancestral reference population is the combined zoo populations that were used in the initial phases of the reintroduction. Thus, inbreeding levels refer to inbreeding that accumulated since the zoo populations.

Inbreeding and population dynamics

To investigate the effects of inbreeding on population dynamics we conducted multiple regressions in the software package R with density dependence or population growth rate as dependent variables. In both regression models inbreeding and hunting intensity were the explanatory variables. Hunting intensity was calculated as the number of hunted individuals since harvest started, divided by the sum of the census counts over the same time period.

Results

Comparison of the first and last 30 years of the time series

Parameter estimates did not converge for two (fo, rw) and three (al, cf and ma) of the 19 populations for the first and last 30 years of the time series, respectively.

The growth rates of the populations for the first 30 years (mean 0.132) were significantly smaller than for the last 30 years (mean 0.205; t-test: t= ,-2.20 n_1=16 , n_2=17 , p=0.03). However, growth rates were not significantly correlated within populations between the first and last 30 years (Figure 1) (Pearson correlation, r = 0.30, p = 0.33, n=13), in spite of substantial overlap in the time series data between the two time periods for many of the populations. Note, however, that given the relatively small sample size of this comparison, we cannot exclude the possibility that the estimates are slightly correlated.

Parameter estimates of the last 30 years

The proportional variance component due to the underlying process noise (Φ_1) had a mean of 0.756 with a range of 0.253 to 0.941 among the populations. In 14 of the 16 populations for which the analysis converged, the mean estimate of the posterior distribution of density dependence was negative indicating density dependence (Table 1). However, the estimates were small and in only three (ch, ju, wb) populations did the 95% confidence interval not include zero. The mean value of b was -0.0021 if only the three populations with significant density dependence were included. As expected with models of this kind, parameter estimates were correlated (environmental stochasticity and observation error: r=0.40, growth rate and density dependence: r=-0.71) but these values are comparatively low suggesting that the models were not overparametrized (Draper & Smith 1981, p. 488).

Inbreeding and population dynamics

Mean inbreeding among the 19 populations was 0.139 with a range from 0.062 to 0.223 among the populations (Table 1). Inbreeding did not have a significant impact on density-dependence in the multiple regression analysis (Table 2). Hunting intensity marginally influenced density dependence (Figure 2) with less pronounced density-dependence at higher hunting pressures. In contrast to density-dependence, population growth rates were marginally affected by inbreeding levels and not by hunting intensity (Table 2). The more inbred a

population was, the less was the population growth rate (Figure 3). Inbreeding explained 12% of the variation in the growth rate among the populations.

Discussion

We found only marginal evidence that inbreeding leads to reduced population growth rates. However, there are reasons to believe that these results may not yet be robust. First, some of the estimated growth rates seem too high. Reasonable maximum rates of increase for this species are thought to be around 0.30 (Loison *et al.* 2002; Toigo *et al.* 2002). Two populations had considerably higher estimated growth rates (fo: 0.34 and wb: 0.42) and may therefore bias the slope of the relationship between inbreeding level and population growth rates (Figure 3). Second, the slope of the regression is likely to be biased downward as the inbreeding estimates (a predictor variable in our models) are likely to contain substantial errors (Draper & Smith 1981, p. 123).

Although there are to date no studies that investigated inbreeding depression in individual fitness in Alpine ibex, the inbreeding levels in Alpine ibex are in the range where other studies have found inbreeding depression (Keller 1998; Reid *et al.* 2003; Fredrickson *et al.* 2007). Even if there is inbreeding depression at the level of the individual in Alpine ibex it may only result in a reduced growth rate if hard selection, e.g. density- and frequency-independent selection is acting (Wallace 1975). In concordance with other studies, we found only weak density dependence (Saether *et al.* 2007), if at all, in the Alpine ibex populations. Weak density-dependence suggests that soft selection may not be the rule in Alpine ibex. This in turn points to the potential that inbreeding depression may lead to reduced growth rates. There is a caveat to this conclusion, however. The precision of the estimates of density dependence is low if the observation error variance is not known correctly (Knape 2008). We do not have empirical estimates of the observation error variance and thus had to estimate it with a state-space model suggesting that our estimates of density dependence might be imprecise.

Besides the potential effect of inbreeding on population growth rates, they are influenced by many other factors. For ungulates in temperate areas, climate

variation is an important factor influencing population fluctuations (Saether 1997; Post & Stenseth 1999; Mysterud *et al.* 2001). Harsh winter conditions might imply soft selection in inbred Alpine ibex populations, if the least inbred individuals are occupying the limited suitable winter habitats. However, inbred individuals might be more affected by harsh winter climate than outbred individuals independent of the population density as inbreeding depression might be more pronounced in stressful environments (Bijlsma *et al.* 2000; Keller *et al.* 2002; Armbruster & Reed 2005). Thus, in winters with unfavourable climate inbred Alpine ibex populations might experience higher losses than less inbred populations. Further studies should investigate this issue as well as the time it takes for relatively inbred and outbred populations to recover from a sudden reduction in population size due to climate or disease outbreaks.

Sampling from different geographic areas may lead to spurious associations between marker genotypes and phenotypes (Lynch & Walsh 1998). In this study, the spurious association would be between inbreeding and population growth rates, when in reality growth rate influenced inbreeding in the early years of the time series when population sizes were small. For alpine ibex, winter climate is one main factor affecting the annual changes in population size (Jacobson *et al.* 2004; Grotan *et al.* 2008). Thus, regions with consistently more snow are likely to have reduced population growth rates compared to regions with less snow irrespective of the inbreeding levels. It seems unlikely that our results are strongly affected by such effects because population growth rates were not correlated between the first and last 30 years of each time series (Figure 1). Populations with low growth rates after founding thus did not necessarily have low growth rates in the last years. While ignoring habitat differences thus seems unlikely to have created a strong spurious association between population growth rates and inbreeding, it may constrain our ability to detect inbreeding depression in population growth rates because environmental effects might obscure the effects of inbreeding on population dynamics. For Alpine ibex some of the known environmental factors with significant effects on population dynamics are snow cover (Jacobson *et al.* 2004; Grotan *et al.* 2008), temperature and precipitation (Grotan *et al.* 2008). Taking some of these environmental factors into account

might improve our ability to detect inbreeding depression in population growth rates.

We found marginal evidence that density-dependence was more pronounced in populations with less hunting pressure. This is in line with a release from density-dependence due to harvest, which in turn increases the recruitment rate of the populations (Proaktor *et al.* 2007). Thus, while hunting intensity may affect the recruitment rate of populations, it generally does not affect the specific deterministic growth rate which we estimated with our models (Saether, pers.comm).

The high values of the variance component due to the process noise suggests that on average 25.6% of the variation in the fluctuations are due to observation error. A relatively low observation error is consistent with a previous study of Swiss Alpine ibex populations (Saether *et al.* 2007). This is not surprising, because ibex can be spotted relatively easily in the treeless habitat by experienced game wardens. Additionally the counts are only carried out in weather with high visibility. However, we found also high variation in the variance component due to process noise among the populations. This variation might be either due to differences in the observation error or environmental stochasticity or both. Different topography among populations may lead to both, variance in the precision of the census error and variance in sensitivity to environmental fluctuations (Wang *et al.* 2009).

This study highlights the difficulty in addressing inbreeding depression in population growth rates in natural populations, particularly in long-lived species. Up to date increased extinction risk due to inbreeding could be shown only in experimental settings (Newman & Pilson 1997; Bijlsma *et al.* 2000) and in short-lived species (Saccheri *et al.* 1998). Extinction or population decline is always the result of several environmental and biological factors (Allendorf *et al.* 2008) and it remains to be seen what impact inbreeding has.

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Table 1: Characteristics of the time-series data of 19 Alpine ibex populations and estimated population dynamic parameters of the last 30 years of the time-series.

Pop	length ts	% missing	hunt. Intens.	r	b	ϕ_1	N	inbr
al	88	2.3	0.14	NA*	NA*	NA*	61	0.111
ap	53	0.0	0.12	0.086	0.0004	0.56	30	0.191
bo	47	0.0	0.06	0.289	-0.0026	0.92	18	0.120
ca	40	5.0	0.07	0.208	-0.0016	0.84	31	0.187
ch	24	0.0	0.04	0.228	-0.0009	0.25	24	0.106
cf	50	12.0	0.08	NA*	NA*	NA*	27	0.154
Fl	41	0.0	0.08	0.189	-0.0005	0.92	32	0.172
fo	46	50.0	0.05	0.335	-0.0010	0.76	27	0.136
gh	97	0.0	0.09	0.116	0.0000	0.94	47	0.113
hw	43	7.0	0.15	0.180	-0.0001	0.92	28	0.153
ju	59	0.0	0.02	0.227	-0.0024	0.39	19	0.141
ma	39	0.0	0.14	NA*	NA*	NA*	22	0.124
ob	39	5.1	0.10	0.158	-0.0005	0.89	30	0.223
pi	47	25.5	0.03	0.187	-0.0019	0.77	17	0.165
rw	49	12.2	0.11	0.179	-0.0002	0.70	29	0.114
sm	84	23.8	0.06	0.130	-0.0003	0.71	32	0.071
um	38	7.9	0.16	0.249	-0.0011	0.93	29	0.114
we	82	9.8	0.00	0.106	-0.0013	0.93	19	0.181
wb	50	0.0	0.07	0.419	-0.0030	0.67	21	0.062

Length ts: length of time series; % missing: percent missing data of the total time series; hunt.intens: hunting intensity; r: population growth rate; b: density dependence; ϕ_1; proportional variance component due to the underlying process noise; N: sample size of genetic data; inbr.: inbreeding estimated with population specific Fst

*analysis did not converge

Table 2: Effects of inbreeding and hunting intensity on population growth rates and density dependence.

dependent variable	adj. R^2	term	F	p	coefficient
population growth rate	0.12	inbreeding	3.82	0.07	-0.93
		hunting intensity	0.11	0.74	-0.16
density-dependence	0.13	inbreeding	0.71	0.42	0.005
		hunting intensity	3.56	0.08	0.011

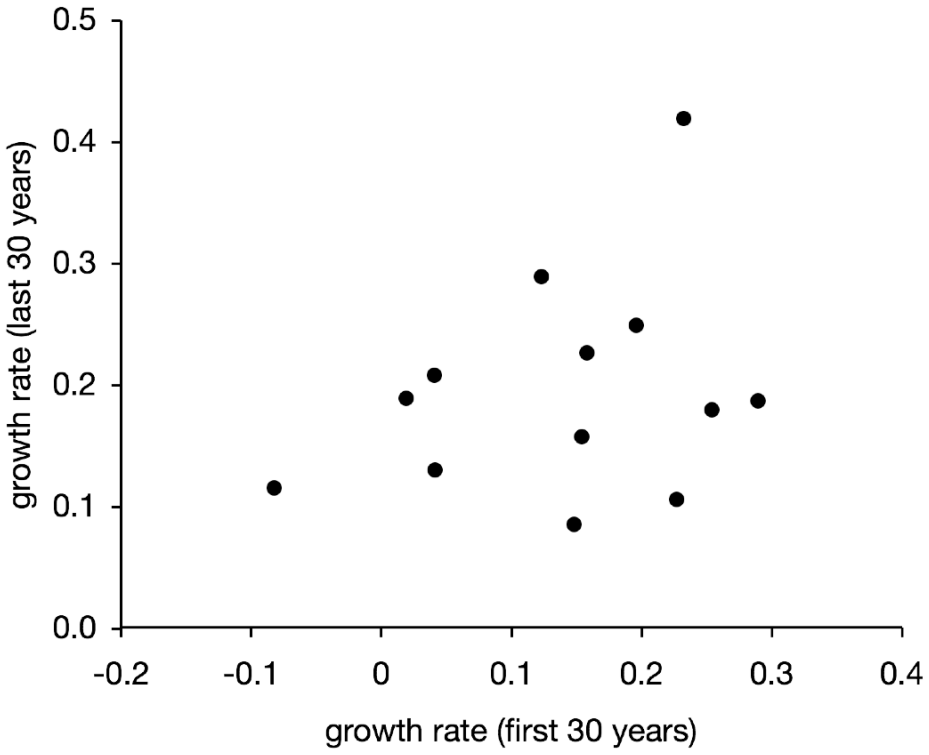

Figure 1: Population growth rates estimated from the first and last 30 years of each time-series were not correlated among 14 Alpine ibex populations. Estimates from five populations did not converge in one of the two time periods.

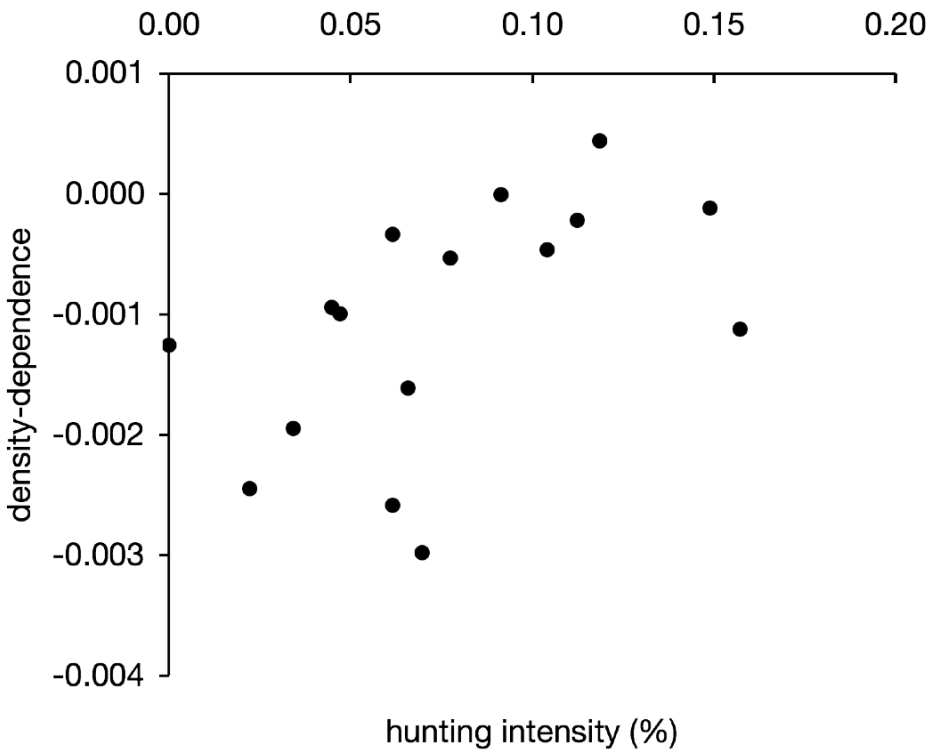

Figure 2: Marginal effect of hunting intensity on density-dependence. Higher hunting intensity reduced the degree of density dependence.

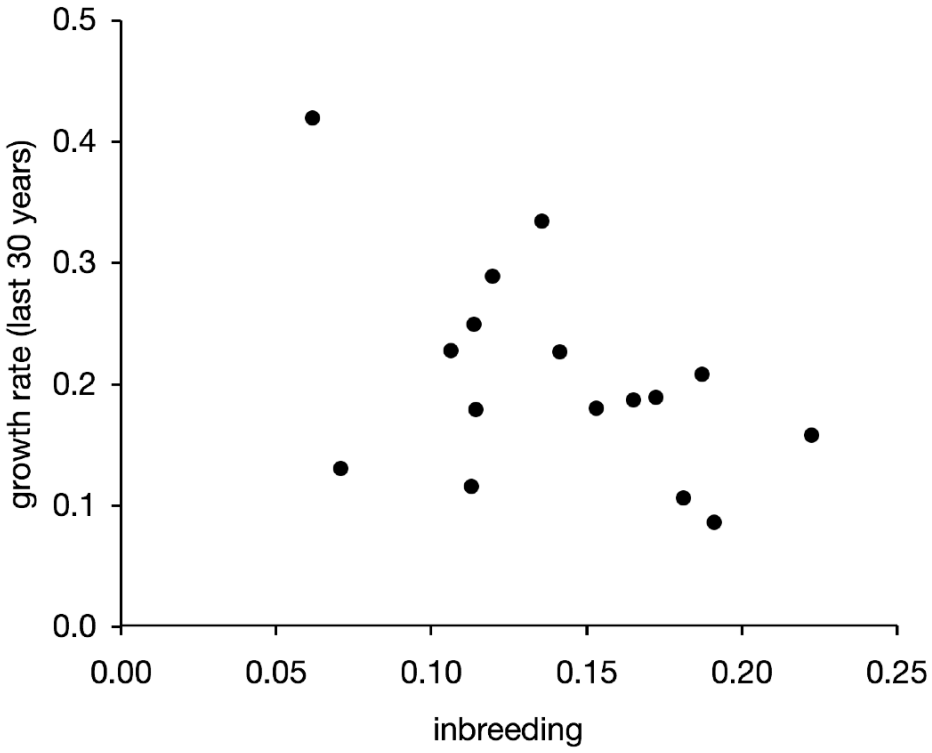

Figure 3: Marginal effect of inbreeding on population growth rates over the last 30 years of the time series. Higher inbreeding levels reduced population growth rates.

PERSPECTIVES

Translocations to reduce inbreeding

This thesis showed that Alpine ibex populations in Switzerland are highly genetically structured, exhibit low levels of genetic variation and high levels of inbreeding. Whether these levels of inbreeding resulted in a reduced population growth rate is not yet clear (Chapter 4) and studies of inbreeding depression in individual fitness in Alpine ibex are lacking. However, data from wild populations suggest that inbreeding depression occurs regularly in plant and animal populations (Keller & Waller 2002). Furthermore, the low genetic variation at the neutral markers suggests that Alpine ibex might have a low potential to adapt to a changing environment (James 1970; Allendorf & Luikart 2007). Thus, also without direct evidence of inbreeding depression it might be prudent to enhance genetic diversity within the Swiss ibex populations to reduce inbreeding and increase evolutionary adaptive potential. Translocating animals from the Italian ancestral population to Swiss populations may not increase genetic variation than translocating animals among Swiss populations because all Swiss populations together had the same genetic variation as the ancestral population (Chapter 1). Furthermore, translocations carry the risk of introducing diseases that have not yet appeared in Switzerland, i.e. Brucellosis (*Brucella melitensis*) or sarcoptic mange (*Sarcoptes scabiei*) (Ferroglio *et al.* 1998; Alasaad *et al.* 2008). Thus, one question emerging from this thesis is how translocations should be best realised to increase genetic variation without undue risk of introducing disease. In addition, limited resources require management concepts to trade off between the benefits and the costs of such translocations. Designing and proposing to the Federal Office for the Environment (FOEN) different possible translocation scenarios is one of the next objectives of this ibex project.

Comparing observed genetic patterns to those expected under neutrality

In this thesis I used information on the demographic history to investigate the effects of the reintroduction history on genetic structure, variation and inbreeding. However, I was not able to capture the whole history in the analyses. Instead, I

had to categorize some historical events and omit some populations that did not fit in these categories (Chapter 1 and 3). Aeschbacher (2007) simulated in his Diploma thesis the trajectory of Swiss ibex populations and the dynamics of neutral loci conditional on the demographic history of these populations. This individual-based simulation tracks pedigrees and genotypes at neutral genetic markers of each individual. Therefore, these simulations are well suited to compare observed genetic patterns to those expected under neutrality. For instance, I will explore to what extent gene flow between neighbouring populations obscured the picture of genetic structure in comparison to pure drift. Although Aeschbacher (2007) already investigated this question, only a subset of the populations was used and the rate of gene flow was assumed constant among all pairs of populations that exchange migrants. Aeschbacher (pers. comm.) has in the meantime developed these simulations further to incorporate individual gene flow rates between population pairs. Therefore, a more precise comparison is now possible.

Inbreeding and population growth rates

My analyses of the effects of inbreeding on population growth rates were inconclusive (Chapter 4). Several aspects of the analysis can be improved to get a clearer picture of this relationship. First, some of the estimated intrinsic rates of increase seem too high for what is known from Alpine ibex (Toigo *et al.* 1996; Loison *et al.* 2002) and large mammals (Caughley 1980) and the models failed to converge in some populations. Solving these non-trivial technical problems and re-estimating the parameters will be one of the first goals. Second, since climate has a large impact on population dynamics in northern ungulates (Saether 1997; Post & Stenseth 1999; Mysterud *et al.* 2001; Jacobson *et al.* 2004), incorporating climate data may decrease the process error in the estimates of population growth rate. Available meteorological data such as snow height, precipitation, temperature and phenology date back for many years and the network of weather stations is sufficiently dense to get local data for most populations. Third, in this thesis I investigated the relationship of inbreeding and growth rates in a two-step

approach, first estimating the growth rates and then relating them to inbreeding levels in a regression analysis. This two-step approach has the disadvantage that errors in the inbreeing estimates lead to a downward bias of the regression coefficients (Draper & Smith 1981). One solution to this problem is to run a joint analysis where the inbreeding effects are included in the hierarchical population dynamics models. Using inbreeding as a latent variable in the time-series models would be another approach to improve these analyses.

Inbreeding at the population and individual level

One reason for estimating inbreeding at population level in this thesis was that inbreeding estimated from neutral genetic markers is expected to better represent genome-wide inbreeding if it is measured at the population level than on the individual level (Vali *et al.* 2008). The combination of pedigree and genetic data from the individual-based simulations will allow me to test this specifically in the case of the Alpine ibex.

At the individual level, the correlation between inbreeding estimated from molecular markers and from pedigrees is generally quite weak (Balloux *et al.* 2004; Slate *et al.* 2004; DeWoody & DeWoody 2005; Aparicio *et al.* 2007), with the exception of scenarios where substantial identity disequilibrium exists. This occurs in very small populations or in breeding systems with non-random mating, e.g. selfing (Balloux *et al.* 2004). However, population structure may also create identity disequilibrium and therefore population level estimates of inbreeding from molecular markers might be more informative about genome-wide inbreeding than individual level estimaes. Thus, I will use the simulations to explore if the demographic history of Alpine ibex history has created sufficient identity disequilibrium to give reliable inbreeding estimates at the population level.

At the same time, it might be valuable to investigate inbreeding at the individual level as well. One advantage of investigating inbreeding at the individual level compared to the population level is the larger sample sizes as each individual will represent one sample and not each population. However, an

analysis of inbreeding depression at the individual level requires individual fitness measurements. Collecting fitness data in Alpine ibex requires a substantial effort as they are long-lived (Nievergelt 1966) and live in inaccessible habitat. However, horn growth of Alpine ibex can be used as a proxy of individual fitness (von Hardenberg *et al.* 2004). Yearly horn growth increments were measured for most of the samples collected from shot animals and thus provide an excellent data set to test if inbreeding affects a correlate of fitness at the individual level. In the absence of an effect of inbreeding on population growth rates, estimating inbreeding effects on individual fitness may help to understand the underlying mechanism: Whether there is inbreeding depression on individual fitness that does not translate into reduced population growth or whether deleterious alleles were purged.

The statistical power to find evidence for inbreeding depression depends on the variation in inbreeding levels within the population (Slate *et al.* 2004). Inbred wolf lineages provide an example where a lack of variation in inbreeding made it impossible to detect inbreeding depression until some lineages were merged thereby creating variance in inbreeding (Fredrickson *et al.* 2007). The same risk might apply to Alpine ibex. All individuals within one population may be inbred at a similar level due to their history of serial bottlenecks. If that is the case, inbreeding depression might be detected only if ibex are translocated among populations (Hedrick *et al.* 2001) and the fitness of admixed individuals and their offspring is monitored. If there is fixation of the genetic load in the recipient population, admixture will lead to genetic rescue. Such heterosis effects after artificial migration have been reported for several small populations (Westemeier *et al.* 1998; Madsen *et al.* 1999; Hogg *et al.* 2006).

Selection and quantitative trait loci

I identified genetic markers that are potentially under selection by calculating Fst as a function of heterozygosity under an infinite allele model with the software FDIST2 (Chapter 1) (Beaumont & Nichols 1996). This method assumes uncorrelated allele frequencies. This assumption is not met in Alpine ibex

populations and I therefore thinned out populations with correlated allele frequencies (Beaumont & Nichols 1996). However, this approach leads to the loss of information: data from those populations that were thinned out are omitted. Thus, I will use the simulations to derive an expectation for neutral markers appropriate for the Alpine ibex demographic history and I will use these data as a basis to identify loci under selection.

Loci that appeared to be under selection were omitted from this thesis because I was interested in neutral genetic variation. However, selected loci are interesting in their own right. For example, ETH10 is known to be associated with growth and backfat in cattle (Li *et al.* 2004) and heterozygous ibex at this locus show reduced parasite burdens as measured by faecal egg counts (Von Hardenberg *et al.* 2007). In the future, I will also investigate the dynamics of loci under selection in Alpine ibex populations.

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THE NEED FOR A BETTER UNDERSTANDING OF INBREEDING EFFECTS ON POPULATION GROWTH

Lukas F. Keller, Iris Biebach, Paquita E.A. Hoeck

Animal Conservation (2007) 10, 286-287

Small populations are inherently at risk of stochastic fluctuations and inbreeding. These risks were recognized nearly 60 years ago (Dowdeswell, Fisher & Ford, 1949) but they did not receive widespread attention until the publication of two seminal text books on conservation biology (Frankel & Soulé, 1981; Soulé & Wilcox, 1980) in the early 1980s. Since then an increasing number of studies has shown that inbreeding depression is pervasive in natural populations (Keller & Waller, 2002), at times even among species that are known to inbreed regularly (Ross-Gillespie, O`Riain & Keller, in press).

Inbreeding depression in individual fitness components is, however, of limited importance to conservation biology unless these reductions in individual fitness translate into reduced population growth rates. That inbreeding can potentially reduce population growth rates and increase extinction risks was demonstrated by influential studies of experimental plant populations (Newman & Pilson, 1997) and a butterfly metapopulation (Saccheri *et al.*, 1998) and, more recently, by studies that demonstrate how experimentally restored immigration rapidly reverses negative population growth rates of inbred populations (Hogg *et al.*, 2006). By demonstrating a link between population size (as a proxy of genetic variation) and population growth rates Reed, Nicholas & Stratton's (2007) study adds to the growing evidence that inbred populations may experience reduced population growth rates.

Small and inbred populations do not always, however, experience reduced population growth rates (Broders *et al.*, 1999). Why would inbreeding depression in individual fitness not always translate into a reduced population growth rate? The strongest argument is that of soft selection (Saccheri & Hanski, 2006). Under many circumstances, the probability of survival of an individual may depend on the presence or absence of other individuals. For example, in a territorial species a proportion of juveniles might die (or emigrate) simply because all territories are occupied by stronger competitors. Had fewer strong competitors existed, all individuals might have acquired territories. That is, the selection is both density- and frequency dependent. Burrowing terminology from international monetary exchange, selection that is both density- and frequency-dependent was coined

'soft selection' by Wallace (1970, 1975). In the context of inbreeding depression, soft selection implies that breeding territories are filled by the least inbred individuals. In a large population these might be outbred individuals but in a very small population these individuals might be appreciably inbred. In the absence of any fitter competitors, these inbred individuals may produce enough offspring so that inbreeding depression in individual fitness has negligible effects on population size (Wallace, 1970, 1975). Thus, if soft selection predominates in natural populations, inbreeding may reduce population growth rates less than individual fitness.

Hard selection, on the other hand, describes selection that is neither density- nor frequency-dependent (Wallace, 1970, 1975). Unconditionally lethal genes are one example of hard selection. If unconditionally lethal genes are a major source of inbreeding depression, then hard selection predominates and inbreeding depression in individual fitness would reduce population size. Despite their importance, we have few estimates of the numbers of unconditionally lethal genes in natural populations. Two estimates from wild fish species suggest, however, that this number is relatively small (1-2 per individual) and comparable to estimates from laboratory studies on Drosophila and Xenopus laevis (McCune *et al.*, 2002). Thus, while some unconditionally lethal genes will undoubtedly be involved, purging is likely to remove such lethals from many populations and a substantial part of inbreeding depression in individual fitness traits is expected to be caused by genes of minor effect (Willis, 1999). Thus, the evidence we have on the genetic architecture of inbreeding depression to date suggests that hard selection is not an unevitable consequence of inbreeding. Note also, that in the context of structured populations or metapopulations, soft selection represents population regulation at the level of the local subpopulation while hard selection represents regulation at the metapopulation level (Saccheri & Hanski, 2006; Whitlock, 2002).

These considerations allow us to predict under which circumstances we would expect inbreeding depression in individual fitness to translate more strongly into reduced population growth rates. For example, populations which

exhibit low levels of density-dependence and those that form part of a metapopulation, are expected to show stronger effects of inbreeding on population growth rates, as would populations with higher genomic numbers of unconditional lethals. The study of Saccheri *et al*. (1998) fits this expectation, since the Glanville fritillary butterflies on Åland form part of a metapopulation that is regulated at the metapopulation level. The two spider species studied by Reed *et al.* (2007), on the other hand, do not seem to fit the expectations: there was evidence for density-dependence in survival. However, the populations of *Rabidosa rabida* exchange up to 1.5 migrants per generation suggesting that they might form part of a metapopulation. If this conjecture should be true and if these populations are regulated at the metapopulation level, these aspects of the spiders' biology may explain the evidence for reduced population growth rates among the smaller populations. Estimates of the magnitude of inbreeding depression and of the details of the processes that regulate these spider populations are required to explain the observed effects in detail.

Reed *et al*.'s (2007) study highlights another important aspect: that inbreeding and environmental effects may interact in their effects on population dynamics. That is, population size (as a proxy of genetic variation) affected population growth rates the most in years when prey availability was decreasing. Such synergistic effects of inbreeding and environmental stressors on extinction probabilities are well known from laboratory experiments with Drosophila (Bijlsma, Bundgaard & Boerema, 2000) and from theoretical models (Tanaka, 1998). The likelihood of such synergistic interactions may well turn out to depend on the soft-hard selection continuum, too. The two studies mentioned above suggest this tantalizing conclusion: The models imposed hard selection in that inbreeding affected population growth rates directly (Tanaka, 1998). And in Drosophila, some lethal genes are known to be expressed only under certain environmental conditions (Vermeulen & Bijlsma, 2004), suggesting that environmental stressors can change the genetic architecture of inbreeding depression to one favouring hard selection.

Much of this is still conjecture because we lack data on many of the relevant variables, particularly in natural populations. Defining the conditions under which population growth rates are depressed by inbreeding will remain one of the major challenges for conservation genetics today.

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SUMMARY/ZUSAMMENFASSUNG

Summary

At the end of the 19th century all large-sized ungulates, except the chamois, were extinct in most parts of Switzerland. While some species came back on their own, Alpine ibex were re-established by reintroductions. Reintroduction programmes generally have to work with relatively few individuals thus creating bottlenecks and founder events. These demographic events create genetic drift, which in turn leads to genetic differentiation between populations, loss of genetic diversity and inbreeding. In this thesis I used independent information of the reintroduction history to assess the impact of this demographic history on the genetics of Alpine ibex populations, using 37 neutral microsatellite loci.

Early in the 19th century only one small population of Alpine ibex survived in northern Italy, which became the ancestral population to all colonies that were re-established across the Alps. Prior to reintroductions into wild habitat, ibex in Switzerland experienced a bottleneck during a captive breeding program in two zoos. Two more bottlenecks followed as a consequence of the initial reintroductions into wild habitat from the zoos (captive-founded) and through founding of new populations from these first populations established in the wild (wild-founded).

Chapter 1 revealed that the reintroduction history left a strong footprint in the present genetic structure of ibex populations. Phylogenetic analyses based on neighbour-joining and clustering algorithms identified three major clades of captive-founded populations together with their corresponding descendant populations. Analysis of molecular variance showed that two thirds of the genetic variation among populations was created by the reintroduction history. All Swiss populations taken together had similar levels of genetic variation as the ancestral population, however each Swiss population by itself had less genetic variation most likely due to genetic drift. The study of serial bottlenecks showed that there was a loss of expected heterozygosity and, therefore, genetic drift with each bottleneck, even when no loss in the number of alleles was observed.

The influence of the founding history on genetic variation was explored in more detail in **chapter 2.** By estimating the number of genetic founders, I found that survival and/or reproduction of the released founder individuals differed between the populations. The number of genetic founders was a better predictor of the genetic variation in a population than the number of released founders. Admixture of the founder group, a measure of the diversity of sources used in a reintroduction, had a positive effect on genetic variation whether measured as expected heterozygosity or number of alleles. However, founder group size had differential effects on expected heterozygosity and number of alleles. While number of founders had a positive impact on number of alleles, it did not have a significant effect on expected heterozygosity. This is in line with the expectation that number of alleles is more sensitive to a bottleneck or founder event than expected heterozygosity. Admixture of the founder group had twice as much influence on number of alleles than the number of founders. Thus, for future reintroductions of species that experienced several founder events, releasing animals from different sources might be more important than releasing many animals from a single source.

The demographic history of populations not only affects genetic variation but also inbreeding. This topic is studied in **chapter 3.** Inbreeding was decomposed into contemporary inbreeding and inbreeding that accumulated over the population history. Contemporary inbreeding, i.e. the inbreeding that is added each generation to the existing inbreeding levels, was estimated by the effective population size. The average ratio of effective population size to census size was similar to the magnitude reported from other mammals. Inbreeding that accumulated over the population history was measured with a population specific Fst which quantifies the accumulation of inbreeding since the subdivision from a common ancestral source. As was the case for genetic variation (chapter 2), the accumulated inbreeding was positively influenced by admixture and size of the founder group. Furthermore, as expected from theory, accumulated inbreeding was affected by the harmonic mean population size since founding of the populations. Higher harmonic mean population sizes translated into less

inbreeding since subdivision. However, contemporary inbreeding and accumulated inbreeding were not correlated. Thus, current rates of inbreeding do not reflect inbreeding that accumulated during the population history. Therefore, current rates of inbreeding cannot be used to predict total inbreeding levels in recently introduced populations.

In **chapter 4** I addressed the question whether inbreeding translates into reduced growth rates of the populations. I estimated growth rates and density dependence with a state-space model in a Bayesian framework. I did not find a clear effect of inbreeding on population growth rates. However, there was a trend towards reduced growth rates among populations with higher inbreeding. Inbreeding depression in individual fitness may not result in a reduced population growth rate if selection is density- and frequency-dependent. I found little evidence for density dependence indicating the potential that inbreeding depression could lead to reduced growth rates in Alpine ibex populations. Density-dependence was marginally more pronounced in populations with less hunting pressure, while population growth rate was unaffected by hunting intensity.

In conclusion this thesis demonstrates a strong influence of the reintroduction history on all genetic parameters investigated. Genetic structure as well as genetic variation and inbreeding were influenced by the demographic history experienced by the populations. My results contribute to a more thorough understanding of how demography is affecting genetic variation and inbreeding especially in bottlenecked populations. This information is valuable for conservation and management in a world with an increasing number of endangered populations and species.

Zusammenfassung

Am Ende des 19. Jahrhunderts waren in den meisten Teilen der Schweiz alle grossen Huftiere mit Ausnahme der Gämse ausgestorben. Während sich die Rehe durch Einwanderung aus Nachbarländern wieder ansiedelten, wurden der Alpensteinbock und der Rothirsch durch Aussetzungen wieder eingebürgert. Diese Wiederansiedlung der verschiedenen Populationen konnte nur mit einer relativ geringen Anzahl von Individuen durchgeführt werden, was zwangsläufig zu Gründereffekten und einer zeitlich begrenzten, sehr geringen Populationsgrösse, einem so genanntem Flaschenhals führte. Diese demographischen Ereignisse haben genetische Drift zur Folge, die wiederum zu genetischer Differenzierung, Verlust von genetischer Vielfalt und Inzucht führt. In dieser Dissertation benutzte ich unabhängige Informationen der Aussetzungsgeschichte, um die Effekte der demographischen Geschichte auf die Genetik der Alpensteinböcke zu erfassen. Zur Beschreibung der Genetik der Alpensteinbockpopulationen habe ich 37 neutrale Mikrosatelliten verwendet.

Zu Beginn des 19ten Jahrhunderts überlebte lediglich eine kleine Population der Alpensteinböcke in Norditalien und wurde damit in der Folge zur Ahnenpopulation sämtlicher wieder angesiedelter Kolonien im Alpenraum. Vor der Wiederansiedlung im natürlichen Lebensraum wurden wenige Tiere der Stammpopulation im Rahmen eines Zuchtprogrammes in Schweizer Zoos vermehrt. Dadurch durchlief diese Zuchtpopulation einen ersten Flaschenhals. Zwei weitere Flaschenhälse folgten, als relativ wenige der gezüchteten Tiere aus den Zoos zur Gründung von Populationen im natürlichen Habitat im Freiland verwendet wurden. Schliesslich wurden wiederum wenige Tiere aus diesen Populationen zur Gründung weiterer Populationen im Freiland umgesetzt.

Kapitel 1 zeigt, dass die Wiederansiedlungsgeschichte einen starken Einfluss auf die gegenwärtige genetische Struktur der Alpensteinböcke hinterlassen hat. Aufgrund von phylogenetischen Analysen, die sowohl auf neighbour-joining als auch auf Cluster-Algorhythmen basieren, stellten sich drei Hauptgruppen von Populationen heraus. Diese drei Hauptgruppen entsprechen den 3 Populationen,

die von Tieren der Zoos gegründet wurden, sowie ihren jeweils abstammenden Populationen. Analysen der molekularen genetischen Varianz ergab, dass zwei Drittel der genetischen Vielfalt zwischen den Populationen aufgrund der Wiederaussetzungsgeschichte entstand. Alle Schweizer Populationen zusammengenommen hatten ähnliche Niveaus der genetischen Vielfalt wie die ursprüngliche Population, wohingegen jede Schweizer Population für sich gesehen eine geringere genetische Vielfalt aufgrund von genetischer Drift aufwies. Die Betrachtung von aufeinander folgenden Flaschenhälsen zeigte einen Verlust von Erwarteter Heterozygotie, und demzufolge auch genetische Drift mit jedem Flaschenhals, auch wenn kein Verlust in der Anzahl Allele zu verzeichnen war.

Der Einfluss der Gründergeschichte der verschiedenen Populationen auf die genetische Vielfalt wurde genauer in **Kapitel 2** untersucht. Indem die Anzahl der genetischen Gründertiere geschätzt wurde, konnte ich sagen, dass sich das Überleben und/oder die Reproduktion der freigelassenen Gründertiere in den einzelnen Populationen unterschied. Die Anzahl der genetischen Gründertiere konnte die genetische Vielfalt innerhalb einer Population besser voraussagen als die Anzahl der freigelassen Tiere. Durchmischung in der Gründergruppe (ein Mass für die Vielfalt der Quellpopulationen) hatte einen positiven Effekt auf die genetische Vielfalt, egal ob sie mit Erwarteter Heterozygotie oder Anzahl Allele gemessen wurde. Jedoch hatte die Gründergruppengrösse einen unterschiedlichen Effekt, je nachdem ob die genetische Vielfalt mit der Erwarteten Heterozygotie oder mit der Anzahl Allele gemessen wurde. Obwohl die Anzahl der Gründertiere einen positiven Einfluss auf die Anzahl Allele hatte, konnte ich keinen signifikanten Effekt auf die Erwartete Heterozygotie feststellen. Dies entspricht der Erwartung, dass die Anzahl Allele stärker auf einen Flaschenhals oder ein Gründungs-Ereignis reagiert als die Erwartete Heterozygotie. Eine durchmischte Gründergruppe hatte doppelt soviel Einfluss auf die Anzahl Allele wie die Anzahl der Gründertiere. Folglich wäre es für zukünftige Wiederaussetzungen von Arten, die mehrere Gründungsereignisse durchlaufen haben, vorteilhafter, wenn mehre

Individuen von verschiedenen Quellen freigelassen werden als viele Tiere von nur einer Quelle.

Die demographische Geschichte der Populationen beeinflusst nicht nur die genetische Vielfalt, sondern auch die Inzucht. Dieses Thema wurde in **Kapitel 3** untersucht. Die Inzucht wurde aufgeteilt in heutige Inzucht und Inzucht, die sich während der Populationsgeschichte angesammelt hat (angehäufte Inzucht). Die heutige Inzucht wurde mit Hilfe der Effektiven Populationsgrösse geschätzt, und bezieht sich auf aktuelle Inzuchtraten, also Inzucht, die bei jeder Generation zu der bereits bestehenden Inzucht hinzukommt. Das durchschnittliche Verhältnis von effektiver Populationsgrösse zur Bestandsgrösse hatte einen ähnlichen Wert, wie man es von anderen Säugetieren kennt. Angehäufte Inzucht wurde mit populationsspezifischen Fst gemessen, das die Ansammlung der Inzucht seit der Aufspaltung von einer gemeinsamen ursprünglichen Population quantifiziert. So wie es bei der genetischen Vielfalt der Fall war (Kapitel 2), wurde die angehäufte Inzucht durch die Durchmischung und Grösse der Gründergruppe positiv beeinflusst. Darüber hinaus war die angehäufte Inzucht vom harmonischen Mittelwert der Populationsgrösse über die Zeit seit der Gründung beeinflusst, so wie es theoretisch zu erwarten war. Eine grössere harmonische Durchschnitts-Populationsgrösse führte zu weniger angehäufter Inzucht. Dennoch bestand keine Korrelation zwischen der heutigen Inzucht und der angehäuften Inzucht. Demzufolge spiegeln die heutigen Inzuchtraten nicht die Inzucht wieder, die sich während der Populationsgeschichte angehäuft hat. Deshalb können die heutigen Inzuchtraten nicht dazu benutzt werden Vorhersagen über die gesamten Inzuchtgrade in kürzlich ausgesetzten Populationen zu treffen.

In **Kapitel 4** habe ich untersucht, ob Inzucht zu einer Abnahme der Wachstumsrate von Populationen führte. Wachstumsrate und Dichteabhängigkeit wurden mit Hilfe eines state-space Modells, gemäss der Theorie nach Bayes, geschätzt. Ich konnte keinen klaren Effekt der Inzucht auf die Populationswachstumsraten feststellen. Jedoch war ein Trend festzustellen, der

reduzierte Wachstumsraten in Populationen mit hoher Inzucht zeigte. Inzuchtdepression auf individueller Fitness führt nicht zwingend zu reduzierter Populationswachstumsrate. Dies kann dann geschehen wenn die Selektion dichte- und frequenzabhängig ist. Ich fand lediglich geringe Anzeichen von Dichteabhängigkeit, die anzeigen, dass Inzuchtdepression zu reduzierten Wachstumsraten in Alpensteinbockpopulationen führen könnte. Dichteabhängigkeit war bei Populationen mit geringerer Jagdintensität ausgeprägter, jedoch war die Populationswachstumsrate nicht durch die Jagdintensität beeinflusst.

Zusammenfassend zeigen diese Untersuchungen einen starken Einfluss der Wiederaussetzungsgeschichte auf alle untersuchten genetischen Parameter. Sowohl genetische Strukturen als auch genetische Vielfalt und Inzucht wurden durch die durchlebte demographische Geschichte der Populationen beeinflusst. Die Ergebnisse steuern zu einem besseren Verständnis bei, inwieweit Demographie die genetische Vielfalt und die Inzucht besonders bei Flaschenhals-Populationen beeinflusst. Diese Informationen sind in einer Welt, in der die Anzahl von bedrohten Populationen und Arten immer weiter ansteigt, wertvoll, um Massnahmen des Artenschutzes Ziel führend und langfristig erfolgreich durchführen zu können.

ACKNOWLEDGMENTS

This thesis would not have been written without the untiring help and support from many people. In my attempt to acknowledge them I will probably forget one or the other. To those, I extend my apologies and thank them no less than had they been named.

Special thanks to Lukas Keller for giving me the opportunity to do this project and being a great supervisor. He always provided support by introducing population genetics to me, giving me shooting lessons, joining me at field work, arranging working retreats in Seraplana and finally taking his valuable time to improve the manuscripts. I very much appreciate that I could attend several workshops, conferences and do fieldwork in Galapagos, a place without ibex, but lovely Mocking birds instead.

I would like to thank the people in our group, past and present members, for creating such a pleasant working atmosphere. Special thanks to Barbara Oberholzer for typing the pile of field protocols into the computer and for combing through all the historical documents, Thomas Bucher for great support in the laboratory, Simon Aeschbacher for compiling much of the ibex history, Peter Wandeler and Erik Postma for helpful discussions, advice and comments on the manuscripts, Paquita for taking me as a field assistant and Christine for her support on professional and personal level, for her assistance in the field and for keeping the friendship even after she had left our group.

Special thanks to Heinz Maag for patiently changing the biopsy darts until we had the prototype and for producing many biopsy darts.

Many thanks to all the game wardens for collecting the samples. I appreciate to get to know some of them and to learn a lot about ibex and nature. The field days were only a small part of the work of the thesis, however, they were a main reason why I enjoyed the time during the PhD so much. Listing all game wardens, who contributed would be too far-reaching. Nonetheless, I would like to mention and thank the following people. The ibex-capture-crew from Oberwallis for their

hospitality, enjoyable field days and support in the biopsy sampling: Urs Zimmermann, Astrid Zimmermann, Helmut Anthamatten, Alexa Anthamatten, Martin Brantschen, Ludwig Imboden and Josi Theler. Those, who invested time and provided support for biopsy sampling: Bruno Dauwalder, Hubert Käslin, Toni Schmid, Paul Schmid, Christian Siegenthaler. Those, who I was able to join in the field: Niklaus Flütsch, Kurt Gansner, Daniel Godli, Martin Gujan, Heinz Guler, Toni Infanger, Karl-Heinz Jäger, Pep Schaniel, Thomas Wehrli. As well many thanks to Meieli and Hans Grossen for the warm hospitality when I sampled around Kandersteg.

I would like to thank the hunting agencies and the Steinbocksteuergruppe for their great support, in particular Guido Ackermann, Kurt Antener, Jürg Brosi, Erwin Eggenberger, Sabine Herzog, Nicole Imesch, Hannes Jenny, Sepp Muggli, Reinhard Schnidrig, Sepp Walker, Urs Zimmermann, Martin Zuber

I thank Mark Kéry and Michael Schaub for their speedy help with the statistics for the population dynamics analysis.

Further thanks to Achaz von Hardenberg and Bruno Bassano to provide samples from the Gran Paradiso Nationalpark.

Thanks to the Federal Office for the Environment (FOEN) and the University of Zurich for their financial support of this project.

I am very grateful to my friends who patiently accompanied me during the time of the PhD. Special thanks to Annina Meier-Schriber for looking after me and keeping me happy with delicious meals. I would like to thank my parents Brigitte and Herbert Biebach, my sisters Anne Biebach and Britta Biebach and my loving partner Frank Reinisch for all their support and love.

Finally, I thank all the lovely Alpine ibex, who gave me the motivation to continue the laboratory and computer work. I hope this thesis can contribute a bit to a healthy future of them.

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